ATMOSPHERIC DEPOSITION OF NITROGEN AND NITROGEN CYCLING: LINKS TO THE SMALL-MAMMAL COMMUNITY

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Submitted to the Faculty
of the Graduate College of
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2003

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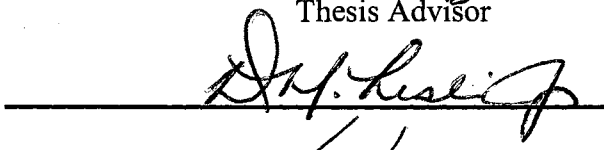

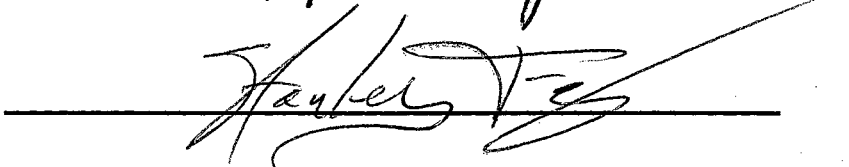

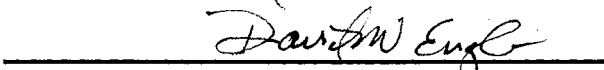

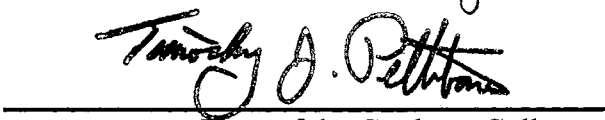

Dean of the Graduate College

DEDICATION

To my wife, Stacy. I love you!

PREFACE

All chapters of this dissertation were written as manuscripts that will be submitted to peer-reviewed journals. Therefore, each chapter follows the style and guidelines of the respective journal in which it was intended to be submitted.

ACKNOWLEDGMENTS

Effective research is dependent on the collaborative efforts of numerous individuals, thus, there are many folks to thank. First, I would like to thank my major advisor Dr. Eric Hellgren for giving me the opportunity to work on this project. We developed a personal relationship over the last 4 years that allowed us to wax philosophically about work, sports, and life. Eric was always available and very open-minded to my thoughts and ideas (which does not necessarily mean we agreed with each other all the time). He maintained a perfect balance of providing sound guidance while still allowing me independence to implement and pursue my own ideas. I have benefited greatly from having him as an advisor and friend.

My committee members, Drs. David (Chip) Leslie, Jr., Dave Engle, and Stan Fox provided support in many ways. I am grateful for their wise counsel and willingness to assist me in my research and professional development. I owe a special thanks to Chip for providing logistical support and his encouragement and support of my professional activities. Dr. Eric Jorgensen (EPA) was a major contributor to the project and was always willing to provide logistical and technical support when needed. Additionally, I appreciate the contributions of the late Dr. Robert Lochmiller which were important to the genesis of this project.

Being able to depend on numerous technicians to collect quality data is extremely important and was vital in conducting this research. They made the field and lab work much more enjoyable and were patient with me in my times of frustration and despair. I

am indebted to Jeff Lindsey, Perry Hanson, Larry Levesque, Matt Bahm, Jesse Bahm, Matt Leslie, Kyle Brazil, Ben Bickerstaff, and Chad Powers for their efforts and friendship. Although a long list, each of these guys significantly contributed to this research in a variety of ways. Matt Bahm worked with me for >1.5 years and I am especially grateful for his dedication and willingness to work.

The Oklahoma Cooperative Fish and Wildlife Research Unit is blessed with an extraordinary staff: Chip, Judy Gray, Sheryl Lyon, and Helen Murray. They made the logistics of this research much easier to deal with and were always willing to help at a moment's notice.

One of the aspects of graduate school that I enjoy the most is meeting and making great friends. I am extremely grateful to all the friends I have made while at OSU. They were always there to lend advice, offer a helping hand, or share a cold one. The list of names is way too long to include here, but please know I cherish the memories and look forward to catching-up in future encounters.

My parents, Jonathan and Barbara Clark, have always encouraged me to chase my dreams. Without their support and understanding, I would not be where I am today. They have had a profound influence in my life, and I am incredibly lucky to be their son. The encouragement, prayers, and support of my sister, Christy, and her husband and son are greatly appreciated as well.

Finally, I owe everything to Stacy. She is my lifeblood, best friend, and soul mate. Her willingness to marry me, move to Oklahoma, and put up with my crap is beyond me! God has truly blessed me with a wonderful wife and I am forever grateful. Without your love, I am much less of a man.

Funding for this project was provided by the United States Environmental Protection Agency (EPA) under an interagency agreement with the United States Geological Survey's Biological Resources Division and administered by the Oklahoma Cooperative Fish and Wildlife Research Unit (Oklahoma State University, Oklahoma Department of Wildlife Conservation, United States Geological Survey, and Wildlife Management Institute cooperating). Additional funding was provided through a fellowship from the Environmental Institute at Oklahoma State University.

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CHAPTER I

POPULATION DYNAMICS OF HISPID COTTON RATS (*SIGMODON HISPIDUS*) ACROSS A NITROGEN-AMENDED LANDSCAPE

Abstract: Population dynamics of some small-mammal species appear to be regulated by plant-community structure, vegetation cover, plant diversity, and food quality. Thus, changes in the plant community associated with nitrogen amendments would likely affect dynamics and structure of small-mammal populations. We conducted a mark-recapture experiment to examine population dynamics of hispid cotton rats (*Sigmodon hispidus*) in response to low-level nitrogen amendments (16.4 kg N/ha/yr) in an old-field grassland. The experimental design consisted of 16, 0.16-ha plots with 4 replicates of each treatment combination (fenced, nitrogen amendment; unfenced, nitrogen amendment; fenced, control; unfenced, control). We predicted that densities, reproductive success, movement probabilities, and survival of cotton rats would be greater on nitrogen-amended plots because of greater aboveground biomass and cover. Population densities of cotton rats tended to be highest on nitrogen-fenced plots, but densities on unfenced-nitrogen plots were similar to control and fenced plots. We observed no distinct patterns in survival, reproductive success, or movement probabilities with regard to nitrogen treatments. However, survival and reproductive success tended to be higher for cotton rats on fenced plots compared with unfenced plots and likely was attributed to decreased predation on fenced plots. As low-level nitrogen amendments continue to be applied, we predict survival, reproduction, and population growth rates of cotton rats on control plots, especially fenced plots with no nitrogen amendment, will

eventually exceed those on nitrogen-amended plots as a result of higher plant species diversity, food availability, and better quality cover.

Introduction

Humans have modified the global nitrogen cycle to the extent that we annually fix more nitrogen than all natural pathways combined (Vitousek 1994, Vitousek et al. 1997*b*). Future atmospheric deposition of nitrogen from anthropogenic activities is expected to increase as global human population and reliance upon fossil fuels increases (Galloway et al. 1994, U.S. Environmental Protection Agency 1995). Thus, the amount of unretained nitrogen cycling through ecosystems is likely to increase and can reasonably be expected to cause future environmental problems. These biogeochemical alterations of the nitrogen cycle could have significant effects on ecological processes at population, community, and ecosystem levels (Vitousek 1994, Chapin et al. 1997, Vitousek et al. 1997*a*, Fenn et al. 1998).

Nitrogen is the primary nutrient limiting terrestrial plant production (Wedin and Tilman 1996). Nitrogen additions can change dominance hierarchies among species and decrease overall biodiversity of ecosystems by altering species composition, species diversity, and structure of food webs (Vitousek et al. 1997*a*, 1997*b*). For example, nitrogen amendments converted heathlands to species-poor grasslands and forest in the Netherlands (Aerts and Berendse 1988), caused shifts from forb- to grass-dominated communities in alpine tundra in Colorado (Bowman et al. 1993, 1995), and may have led to the conversion of slow-growing, slow N-cycling spruce-fir forests to faster growing, fast N-cycling deciduous forests in New England (McNulty et al. 1996).

Nitrogen enrichment also has caused qualitative and quantitative changes in grassland vegetation. These changes resulted in an increase in biomass and decrease in plant species diversity (Grant et al. 1977, Tilman 1987, 1996, Carson and Barrett 1988, Hall et al. 1991). For example, after 9 years of annual nutrient enrichment, Carson and Barrett (1988) reported 3 contrasting types of old-field communities with regard to structure and composition and a decrease in species diversity on nutrient-rich plots. Furthermore, long-term nitrogen amendments in artificially constructed plant communities dominated by C_4 prairie grasses resulted in nitrogen-mediated shifts to C_3 nonnative grasses (Wedin and Tilman 1996). These alterations in plant communities affect trophic relationships and nutrient cycling within the ecosystem.

Causal effects attributed to changes in nitrogen availability in ecosystems are more clearly seen in plant than animal communities. Research exploring effects of nitrogen enrichment on consumer communities is rare (Grant et al. 1977, Anderson and Barrett 1982, Hall et al. 1991). Population dynamics of some small-mammal species appear to be regulated by plant-community structure, plant cover, plant diversity, and food quality (Hall et al. 1991). Thus, plant community changes associated with nitrogen additions would likely affect dynamics and structure of small-mammal populations. As with plants (Tilman 1987, 1988; Wedin and Tilman 1996), small-mammal communities may experience a decrease in species richness and become dominated by a few successful competitors that respond to a plant community modified by increased nitrogen availability.

Our objective was to examine how population dynamics of the hispid cotton rat (*Sigmodon hispidus*), the dominant member of the small-mammal assemblage at our

study site, respond to a landscape altered by low-level nitrogen amendment (16.4 kg N/ha/yr) and exclosure fencing (i.e., constraints on herbivory). If nitrogen amendments had the hypothesized effects on the plant community of our study plots (i.e., increased aboveground live plant mass and canopy cover on nitrogen-amended plots; Tilman 1987, 1988; Wedin and Tilman 1996), then several demographic consequences were possible. We predicted densities, reproductive success, and survival rates of cotton rats would be higher on nitrogen-amended plots because of increased aboveground plant biomass (i.e., enhanced concealment from predators). We also predicted that movements by male cotton rats would be biased toward nitrogen-amended plots. Although exclosures were designed to constrain herbivory (i.e., decrease competition from larger primary consumers), we expected population dynamics to be affected by nitrogen amendments rather than exclosures because densities of larger herbivores (e.g., lagomorphs and artiodactyls) were low at the onset of our study.

Study Area

The field research was conducted at the Environmental Protection Agency's Center for Subsurface and Ecological Assessment Research (CSEAR) near Garr Corner, Pontotoc County, Oklahoma. The study area was an old-field site composed of 16 square 0.16-ha experimental plots surrounded and separated from adjacent plots by a 5-m uninhabitable mowed strip (total area = 3.45 ha; Figure I.1). The area had not been cultivated since 1950 but was heavily grazed during the last half-century before January 1998. Dominant vegetation consisted of early to mid-successional grasses and forbs, including old-field threeawn (*Aristida oligantha*), broomsedge bluestem (*Andropogon*

virginicus), western ragweed (*Ambrosia psilostachya*), and heath aster (*Aster ericodes*). Preliminary sampling of small mammals at CSEAR identified existing populations of mice (i.e., *Reithrodontomys* spp. and *Peromyscus* spp.) and least shrews (*Cryptotis parva*), but cotton rats were absent on our experimental plots before our study (E. E. Jorgensen, unpublished data). This initial absence of cotton rats was not unexpected, because adequate concealment cover was lacking across our experimental plots at the onset of our study. Furthermore, Phillips (1936) had previously reported an absence of hispid cotton rats in overgrazed pastures in central Oklahoma.

The experimental design consisted of 2 treatments (exclosure fencing and nitrogen amendment) randomly applied to the study plots in a 2 × 2 factorial with 4 replicates per treatment (fenced, nitrogen amendment; fenced, control; unfenced, nitrogen amendment; unfenced, control). Fenced plots were surrounded by a 2-m high, 2.5-cm chain-link fence that allowed free movement of small mammals between plots but restricted access by terrestrial predators and larger mammalian herbivores (e.g., lagomorphs, artiodactyls). To ensure free movement of adult cotton rats between plots, 7.5-cm triangular holes were cut at ground level and spaced at 2-m intervals around fenced plots. Beginning in February 1999, we added nitrogen fertilizer (34% ammonium nitrate) to nitrogen-amended plots at a rate of 3.1, 5.1, 4.1, and 4.1 kg N/ha in February, May, August, and November, respectively. Nitrogen amendment rates corresponded to seasonal rainfall proportions for central Oklahoma. Oklahoma receives approximately 10 kg/ha/yr of nitrate via atmospheric deposition (National Atmospheric Deposition Program (NRSP-3)/National Trends Network 2002). Nitrogen amendments of 16.4 kg N/ha/yr were chosen for the study because rates of atmospheric deposition of nitrogen in

excess of this occur in industrialized regions of the globe and are projected to increase for the foreseeable future (Brimblecombe and Stedman 1982, Galloway et al. 1994, U.S. Environmental Protection Agency 1995, Vitousek et al. 1997*b*).

Methods

Vegetation sampling and analyses

We collected aboveground live mass in August 1998, 1999, and 2000. Current year's growth was clipped at the soil surface within 5, 0.1-m^2 quadrats/plot and separated by monocots and dicots. We dried clipped samples at 60°C in a forced-air oven to a constant weight. Samples were weighed and recorded as monocot, dicot, and total mass. We estimated canopy cover in May and September 1999 and 2000 within 25, 0.1-m^2 quadrats per plot using the Daubenmire cover class method (Bonham 1989). Canopy cover was estimated by species and later summarized as monocot, dicot, and total cover.

We tested for pre-treatment differences in monocot, dicot, and total aboveground live mass and canopy cover across our treatment plots using 2-way analysis of variance (PROC MIXED; SAS 2000). Although we did not sample canopy cover before initial nitrogen amendments (February 1999), we used our sampling period from May 1999 to test for "pre-treatment" differences in canopy cover. To test for post-treatment differences, we tested for a nitrogen effect between amended and non-amended plots for total canopy cover and aboveground live mass using 2-way analysis of variance with repeated measures (PROC MIXED; SAS 2000). We fitted a multiple variance model and used the Kenward-Roger approximation to calculate effective degrees of freedom (PROC

MIXED, SAS 2000; Kenward and Roger 1997). We used least-squared means separation tests for all significant main effects with a significance level of $P < 0.05$.

Small-mammal sampling

We sampled small mammals with Sherman live traps (7.6 × 8.9 × 22.9 cm) for 3 consecutive days at 3–5-week intervals from July 1999 to December 2000. Each study plot had 25 traps systematically spaced at 7-m intervals for a potential of 1,200 trap nights/sampling period. Traps were set each afternoon, baited with rolled oats, provided with cotton for warmth during cold weather, and checked between 0600 and 1200 hours. We released captured animals immediately after marking with a unique number via toe clipping. We conducted trapping following standards established by the Animal Care and Use Committee of the American Society of Mammalogists (1998) and operated under Animal Care and Use Protocol 723, Oklahoma State University. We recorded the trap station, species, mass, sex, and reproductive status (scrotal or non-scrotal for males; pregnant, lactating, and open or closed vagina for females) for each individual. Additionally, we recorded instances of accidentally sprung traps (i.e., sprung traps not resulting in capture).

We used minimum number known alive (MNKA; Krebs 1966) as an index to abundance and number of juveniles (≤ 45g) per female as an index to reproductive success for each plot at each sampling period. We compared MNKA and reproductive success between treatment plots using 2-way analysis of variance with repeated measures (PROC MIXED, SAS 2000). We fitted a multiple variance model and used the Kenward-Roger approximation to calculate effective degrees of freedom (PROC MIXED, SAS 2000; Kenward and Roger 1997). We used least-squared means separation

tests for all significant main effects. Because cotton rats were colonizing experimental plots during the first few sampling periods and number of captures for all species was low, statistical comparisons of MNKA and reproductive success were limited to October 1999–December 2000 (i.e., 16 trapping periods). A significance level of $P \leq 0.05$ was used for all analyses.

Population Modeling

We used a multi-strata model (Hestbeck et al. 1991, Brownie et al. 1993) in Program MARK (White and Burnham 1999) to estimate apparent survival, capture probabilities, and movement probabilities of cotton rats across our study plots. Modeling for potential differences in survival between strata was our primary interest, thus we examined models with varying strata effects (i.e., no treatments, nitrogen only, fence only, and combination of fence and nitrogen) for survival. Models were ranked using Akaike's Information Criterion (AICc) and were averaged to determine final parameter estimation using AICc weights (Burnham and Anderson 1998). Our global model (i.e., most parameterized) included sex and strata effects for all parameters and time effects (i.e., nonbreeding and breeding seasons) for survival and recapture probabilities. We used trapping data to determine breeding and nonbreeding seasons and defined an interval between trapping periods as breeding season if ≥10% of females were in reproductive condition (i.e., pregnant, lactating, or open vagina) during the latter trapping period.

We also modeled for differences in transition probabilities of cotton rats between strata with emphasis on male movement. Using the most parsimonious model from the survival analysis, we examined models with varying sex and strata effects for transition

probabilities. Additionally, we examined models accounting for unequal distances between treatment plots by adjusting transition probabilities with the average distance between plot-center of each plot to plot-center of all plots in a different stratum. Transition probabilities were held constant across time in all models.

For all models, we accounted for varying time intervals between sampling periods (3–5 weeks) by adjusting parameter estimates to a 30-day interval between periods. We adjusted capture probabilities for varying sampling effort among strata by accounting for sprung traps (i.e., traps that captured animals and those accidentally sprung) in all models. Lacking specific information on timing of trap-springing, we assumed that each trap was sprung halfway between trap-setting and trap-checking (Nelson and Clark 1973, Beauvais and Buskirk 1999). Thus, sampling effort for each reproductive season was calculated for each stratum as:

$$\frac{\sum_i t_i - (s_i \times 0.5)}{b},$$

where t was the maximum number of potential trap nights within trapping period i (i.e., maximum = 300 trap nights/stratum), s was the number of sprung traps at trapping period i, and b was the maximum number of potential trap nights during a season (i.e., breeding or nonbreeding).

Examination of instantaneous sampling assumption

Similar to the standard Jolly-Seber model, the multi-strata model assumes all samples are instantaneous (i.e., all mortality and movement occurs between, not within, sampling periods; Hestbeck et al. 1991); however, this assumption can never be strictly met (Hestbeck et al. 1991). We examined departures from this assumption by identifying

all occasions when an individual cotton rat was captured in multiple strata within a sampling period. The encounter histories in the original analysis for those individuals were coded by recording the stratum where each cotton rat was initially captured as the encounter for a given sampling period. To investigate violations of the instantaneous sampling assumption, we performed 2 additional analyses with the original candidate set of models to determine if model rankings, identified patterns, and estimates of survival and male movement from the original analysis were robust to departures from this assumption. One analysis (Test-1) was performed by changing the encounter history for every cotton rat captured on multiple strata within a sampling period to the alternative stratum; the other analysis (Test-2) used the same encounter histories from Test-1 but changed the encounter histories for the 9 observations where cotton rats were captured on 3 strata within a sampling period to the third alternative stratum.

Results

Vegetation analyses

We detected no pretreatment differences in monocot, dicot, and total aboveground live mass or canopy cover ($P > 0.2$ for all tests); thus, we assumed homogeneity among experimental plots with respect to aboveground live mass and canopy cover before nitrogen amendments. Although no difference in total canopy cover was observed among treatment plots following nitrogen amendments (nitrogen effect: $F_{1,12.8} = 0.42$, $P = 0.28$; fence effect: $F_{1,12.8} = 0.42$, $P = 0.53$; Figure I.2), we observed a nitrogen effect on total aboveground live mass ($F_{1,17.9} = 7.61$, $P = 0.01$; Figure I.3) but no fence effect ($F_{1,17.9} = 1.46$, $P = 0.24$). Total aboveground live mass was greater on nitrogen-amended

plots than on non-amended plots in 1999 ($t_{35.7}$ = -2.19, P = 0.04) and 2000 ($t_{35.7}$ = -2.24, P = 0.03; Figure I.3); however, nitrogen treatment and year did not interact ($F_{1,24.8}$ = 0.42, P > 0.24).

Small-mammal sampling and modeling

Between July 1999 and December 2000, we recorded 7,955 small-mammal captures in 20 sampling periods (i.e., 24,000 potential trap nights). Cotton rats accounted for 5,468 captures of 982 individuals (males = 538, females = 444) and were the most abundant small-mammal species on our experimental plots.

We observed 2-way interactions for MNKA of cotton rats between the fenced treatment and time ($F_{15, 170}$ = 1.91, P = 0.024) and between nitrogen and fence treatments ($F_{1, 24.3}$ = 10.87, P = 0.003). Tests of effect slices (PROC MIXED, SAS 2000) identified statistical differences across fenced and unfenced plots when amended with nitrogen ($F_{1, 24.3}$ = 31.30, P < 0.001) and across nitrogen and control plots when fenced ($F_{1, 24.3}$ = 21.45, P < 0.001). Abundance of cotton rats tended to be higher on nitrogen-fenced plots ($\bar{x}$ = 18.4, SE = 0.8) compared with other treatment plots (control: $\bar{x}$ = 9.8, SE = 1.0; fenced control: $\bar{x}$ = 11.2, SE = 0.8; unfenced nitrogen: $\bar{x}$ = 9.7, SE = 1.0; Figure I.4). Similarly, we observed a fence effect for reproductive success ($F_{1, 42.8}$ = 4.81, P = 0.03) with higher success on fenced plots (fenced: $\bar{x}$ = 1.5, SE = 0.2; unfenced: $\bar{x}$ = 0.9, SE = 0.1; Figure I.5); however, we observed no evidence to support a nitrogen effect on reproductive success (nitrogen: $\bar{x}$ = 1.3, SE = 0.2; control: $\bar{x}$ = 1.2, SE = 0.2; $F_{1, 42.8}$ = 0.24, P = 0.62).

The minimum AICc multi-strata model provided estimates of survival on fenced and unfenced plots, regardless of nitrogen amendments, for males and females across breeding seasons, and movement for males among all strata with average distance between strata as a covariate (Table I.1). Model-averaged estimates of survival for males and females tended to be higher on fenced plots except during the breeding season in 2000 (Figure I.6). Model-averaged transition probabilities of males between strata ranged from 0.01 (control to fenced control) to 0.10 (fenced control to nitrogen) and showed no clear patterns of movement toward a particular strata (Figure I.7).

Examination of instantaneous sampling assumption

Cotton rats were captured on multiple strata within a sampling period (Table I.2); thus, we evaluated effects of violating the instantaneous sampling assumption by performing 2 additional analyses with the original candidate set of models. The 2 minimum AICc models and respective AICc weights varied across analyses. Model-averaged estimates of survival for males and females during the first breeding season (Breed 1 = 2 August 1999 – 21 November 1999) varied between fenced and unfenced strata (Table I.3), but patterns with respect to the treatments were the same (i.e., higher survival in fenced plots). The percent differences in estimates of survival between the original analysis and Test-1 or Test-2 were ≤ 5.7 % in all other seasons, and patterns with respect to the treatments were the same. Although transition probabilities for males varied between the 3 analyses, probabilities in all models were low (≤ 0.1) and displayed no clear patterns. Thus, we assumed that our original analysis was robust to violations of the instantaneous sampling assumption.

Discussion

Nitrogen amendments to old-field plots at CSEAR caused increases in total aboveground live mass of vegetation during the first year following application (i.e., 1999), but there was no evidence for an effect on canopy cover; thus, vegetation was thicker on nitrogen-amended plots. Similarly, increases in aboveground plant biomass within 1 year of nitrogen amendments were reported for grasslands in Minnesota (Tilman 1987). Although the effect of nitrogen amendments on aboveground live mass was additive (i.e., no interaction with time), aboveground live mass remained greater on nitrogen-amended plots in 2000; thus, testing of our *a priori* predictions concerning changes in population characteristics of cotton rats was valid. Future studies on our experimental plots should account for potential differences in nutritional quality of vegetation (e.g., nitrogen availability in plant foods). However, given the lack of adequate habitat to support cotton rat populations at the onset of our study, our predictions concerning population parameters were based primarily on increased concealment capability (i.e., increased canopy cover or thicker cover to protect from predation) on nitrogen-amended plots.

We did not observe predicted patterns in survival, reproductive success, or transition probabilities regarding nitrogen amendments, although we observed limited evidence for our prediction that cotton rat densities would be higher on nitrogen-amended plots. The combination of increased aboveground live mass and protection from predation on nitrogen-fenced plots likely accounted for differences in population density of cotton rats among the treatment plots. That conclusion was partially supported by

higher survival rates (Figure I.6) and reproductive success (Figure I.5) of cotton rats on fenced plots compared with open plots, regardless of nitrogen additions.

Exclosures that control access to predators have been used to address hypotheses and effects of predation on population characteristics of small mammals (Schnell 1968, Desy and Batzli 1989, Klemola et al. 2000). Exclosures at CSEAR were designed primarily to decrease competition between small mammals and larger herbivores; however, in the absence of a fence effect on vegetation, we attribute differences in survival, abundance, and reproductive success between unfenced and fenced plots to differential predation rather than exclusion of larger herbivores. Although variable across seasons, survival tended to be higher on fenced plots for male and female cotton rats with the most pronounced effect during autumn (September–December; Breed 1 and Non 2, Figure I.7). Similarly, our measure of reproductive success (juveniles/female), which reflects juvenile survival, was higher on fenced plots, regardless of nitrogen additions. Thus, our results for survival, reproduction, and abundance exhibited more of a fence effect than the predicted nitrogen effect.

Predation has regulatory effects on population densities of small mammals (Desy and Batzli 1989, Tait and Krebs 1983, Reid et al. 1995, Klemola et al. 2000). Sign and observations of terrestrial and avian predators, especially coyotes (*Canis latrans*), red-tailed hawks (*Buteo jamaicensis*), and northern harriers (*Circus cyaneus*), were prevalent at CSEAR but not inside fenced plots. The only potential predators observed inside fenced plots were black rat snakes (*Elaphe obsoleta*) and speckled king snakes (*Lampropeltis getula holbrooki*). The most common and frequently observed predator at CSEAR, especially in autumn, was the northern harrier. Cotton rats are common prey of

northern harriers (Carter 1984), and high densities of northern harriers have been associated with high densities of cotton rats (Baumgartner and Baumgartner 1944, Odum 1947). We frequently observed northern harriers hunting across our experimental plots but never observed them landing inside a fenced plot. Northern harriers widely forage and hunt with a low coursing flight frequently pouncing on prey located via visual and acoustical cues (Rice 1982, 1983); thus, size of the exclosures (0.16 ha) and height of the fences (2 m) likely limited access to northern harriers, as well as other aerial predators, and accounted for discrepancies in survival between fenced and unfenced plots during the autumn migration of northern harriers. However, we did not collect empirical data on predator abundance and activity, so our observations were only anecdotal.

Movement probabilities of male cotton rats displayed no clear trends (Figure I.7) and did not support our predictions related to movements toward nitrogen-amended plots. Transition probabilities were relatively low for all strata to strata movements (≤ 0.1) and agreed with other studies that have demonstrated low movement probabilities for cotton rats in fragmented landscapes (Diffendorfer et al. 1995, Diffendorfer et al. 1999). We note that transition probabilities were calculated for strata-to-strata movements (i.e., not plot-to-plot) and held constant across time. Because movement rates of cotton rats may vary temporally (Diffendorfer et al. 1995), a more detailed examination of cotton rat movements incorporating seasonal effects and plot-to-plot movements could provide important information pertaining to dispersal and distribution across our experimental plots relative to nitrogen additions.

Hall et al. (1991) reported changes in populations of meadow voles (*Microtus pennsylvanicus*) resulting from changes in plant community structure, cover, and

diversity after supplementation of nitrogen. Although aboveground biomass was higher on nitrogen-amended plots, non-amended plots had higher plant diversity and quality of cover, resulting in positive population growth rates and higher population densities, rates of recruitment, and survivorship for voles (Hall et al. 1991). Similarly, Anderson and Barrett (1982) reported increased aboveground biomass and decreased plant species diversity on nutrient-amended sites, resulting in decreased densities and survivorship of meadow voles. Although our study incorporated lower levels of nitrogen amendments (16.4 kg N/ha/yr) and was shorter in duration (<2 yr) than the above studies, we still expected to observe increases in aboveground live mass resulting from nitrogen amendments and subsequent effects on small-mammal population dynamics. However, because changes in plant species richness and abundance lag behind changes in aboveground biomass (Tilman 1987, 1988; Wedin and Tilman 1996), we did not expect to observe a detectable decrease in species diversity of plants on nitrogen-amended plots and subsequent shift of the cotton rat population from nitrogen to non-amended plots given the duration of our study. As low-level nitrogen amendments continue to be applied at CSEAR, we predict nitrogen-mediated shifts in the distribution of cotton rats to occur as a result of the cumulative effect of nitrogen amendments and decreased plant species diversity on nitrogen-amended plots. Specifically, we predict that cotton rat densities on control plots, especially fenced plots with no nitrogen amendment, will eventually exceed those on nitrogen-amended plots as a result of higher plant species diversity, food availability, and better-quality cover.

Acknowledgments

This project was funded by the United States Environmental Protection Agency (EPA) under Interagency Agreement DW14938554-01-0 with the United States Geological Survey's (USGS) Biological Resources Division and administered by the Oklahoma Cooperative Fish and Wildlife Research Unit (Oklahoma State University, Oklahoma Department of Wildlife Conservation, USGS's Biological Resources Division, and Wildlife Management Institute cooperating). The views expressed in this manuscript are those of the authors and do not necessarily reflect the views and policies of EPA or USGS. Additional funding was provided through a fellowship from the Environmental Institute at Oklahoma State University. We appreciate the field assistance of J. Lindsey, P. Hanson, L. Levesque, M. Bahm, J. Bahm, M. Leslie, and K. Brazil and logistical assistance of J. Gray, H. Murray, and S. Lyon. The contributions of the late R. L. Lochmiller were important to the genesis of this project. Approved by the Director, Oklahoma Agriculture Experiment Station.

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Table I.1. Model parameters and AICc values for multi-strata models examining survival (*S*) and transition probabilities (*PSI*) of hispid cotton rats (*Sigmodon hispidus*) across a landscape manipulated with nitrogen amendments and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Capture probabilities (*p*) varied by sex and breeding season across all strata in all models.

Model	ΔAICc	AICc weights	Number of parameters	Deviance
{*S*(sex*breeding)FENCE *p*(sex*breeding)ALL *PSI*(male)ALL distance}[a]	0.00	0.90	54	4,030.100
{*S*(sex*breeding)FENCE *p*(sex*breeding)ALL *PSI*(male)ALL}[b]	5.45	0.06	59	4,025.095
{*S*(sex*breeding)FENCE *p*(sex*breeding)ALL *PSI*(sex)ALL distance}[c]	7.46	0.02	60	4,025.010
{*S*(sex*breeding)FENCE *p*(sex*breeding)ALL *PSI*(sex)ALL}[d]	8.28	0.01	69	4,006.892

[a] Survival varies by sex and breeding season for fenced and unfenced plots (i.e., no nitrogen effect) and transition probabilities vary for males across all strata with distance as a covariate and are held constant for females (i.e., no strata effects).

[b] Survival varies by sex and breeding season for fenced and unfenced plots (i.e., no nitrogen effect) and transition probabilities vary for males across all strata and are held constant for females (i.e., no strata effects).

Table I.1. (Continued)

[c] Survival varies by sex and breeding season for fenced and unfenced plots (i.e., no nitrogen effect) and transition probabilities vary by sex across all strata with distance as a covariate.

[d] Survival varies by sex and breeding season for fenced and unfenced plots (i.e., no nitrogen effect) and transition probabilities vary by sex across all strata.

Table I.2. Total individual hispid cotton rats captured and number captured on multiple strata within each sampling period at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000.

Sampling date	Number of individuals captured	Individuals captured on 2 strata	Individuals captured on 3 strata
99 Oct 18	75	11	
99 Nov 21	108	9	
99 Dec 11	139	10	
00 Jan 9	118	3	
00 Feb 6	95	3	
00 Mar 5	107	10	
00 Apr 2	78	11	
00 May 5	107	7	
00 May 22	111	18	2
00 Jun 29	157	22	3
00 Jul 28	235	24	1
00 Aug 20	287	18	1
00 Sep 10	315	12	2
00 Oct 8	348	6	
00 Oct 29	302	10	
00 Dec 3	200	16	

Table I.3. Model-averaged survival estimates from the original analysis and analyses (Test-1 and -2) examining violations to the instantaneous sampling assumption for male and female hispid cotton rats on unfenced and fenced plots during the first breeding season (Breed 1 = 2 August 1999 – 21 November 1999) at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma.

	Survival estimate			% difference between	
Strata	Original	Test-1	Test-2	Original vs. Test-1	Original vs. Test-2
Unfenced					
Male	0.377	0.472	0.466	25.2	23.6
Female	0.671	0.584	0.584	14.9	14.9
Fenced					
Male	0.626	0.570	0.568	9.8	10.2
Female	0.891	0.884	0.884	0.8	0.8

Figure I.1. Layout of nitrogen and exclosure treatments for experimental plots at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000.

Figure I.2. Percent canopy cover of vegetation on control and nitrogen-amended plots in spring (SP) and fall (FA) at the Center for Subsurface and Ecological Assessment Research, Oklahoma, 1999–2000. Error bars represent 1 standard error from the mean.

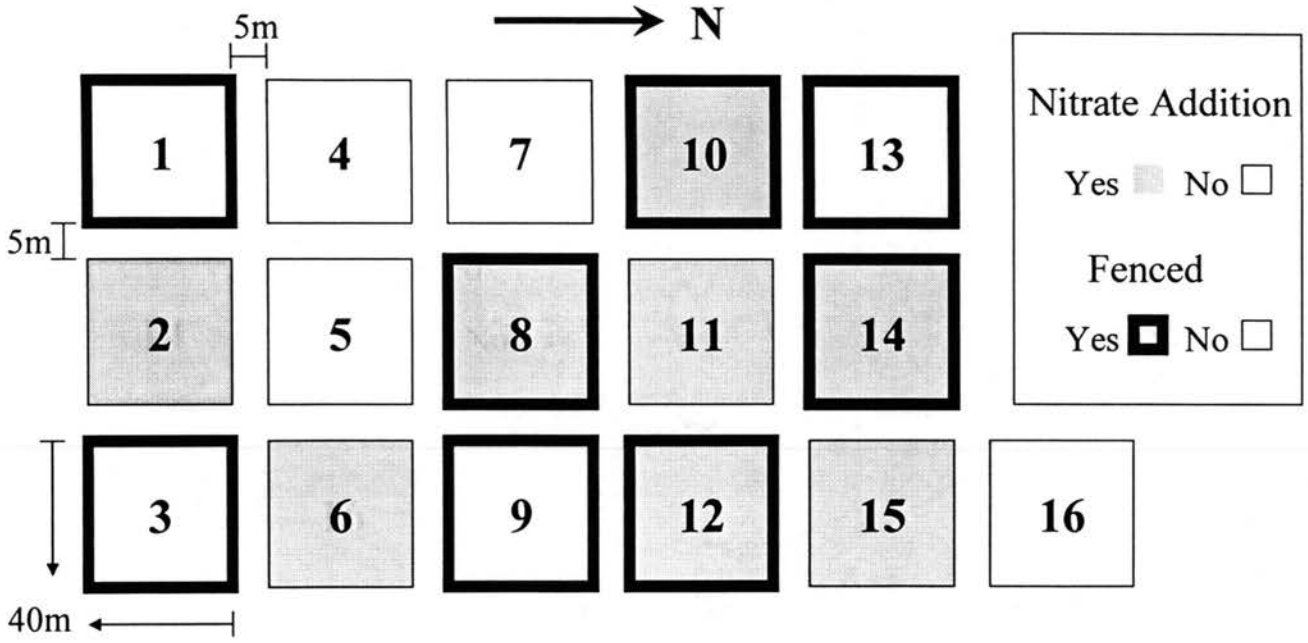
Figure I.3. Total aboveground live mass (g/m^2) of vegetation on control and nitrogen-amended plots at the Center for Subsurface and Ecological Assessment Research, Oklahoma, August 1998–2000. Error bars represent 1 standard error from the mean.

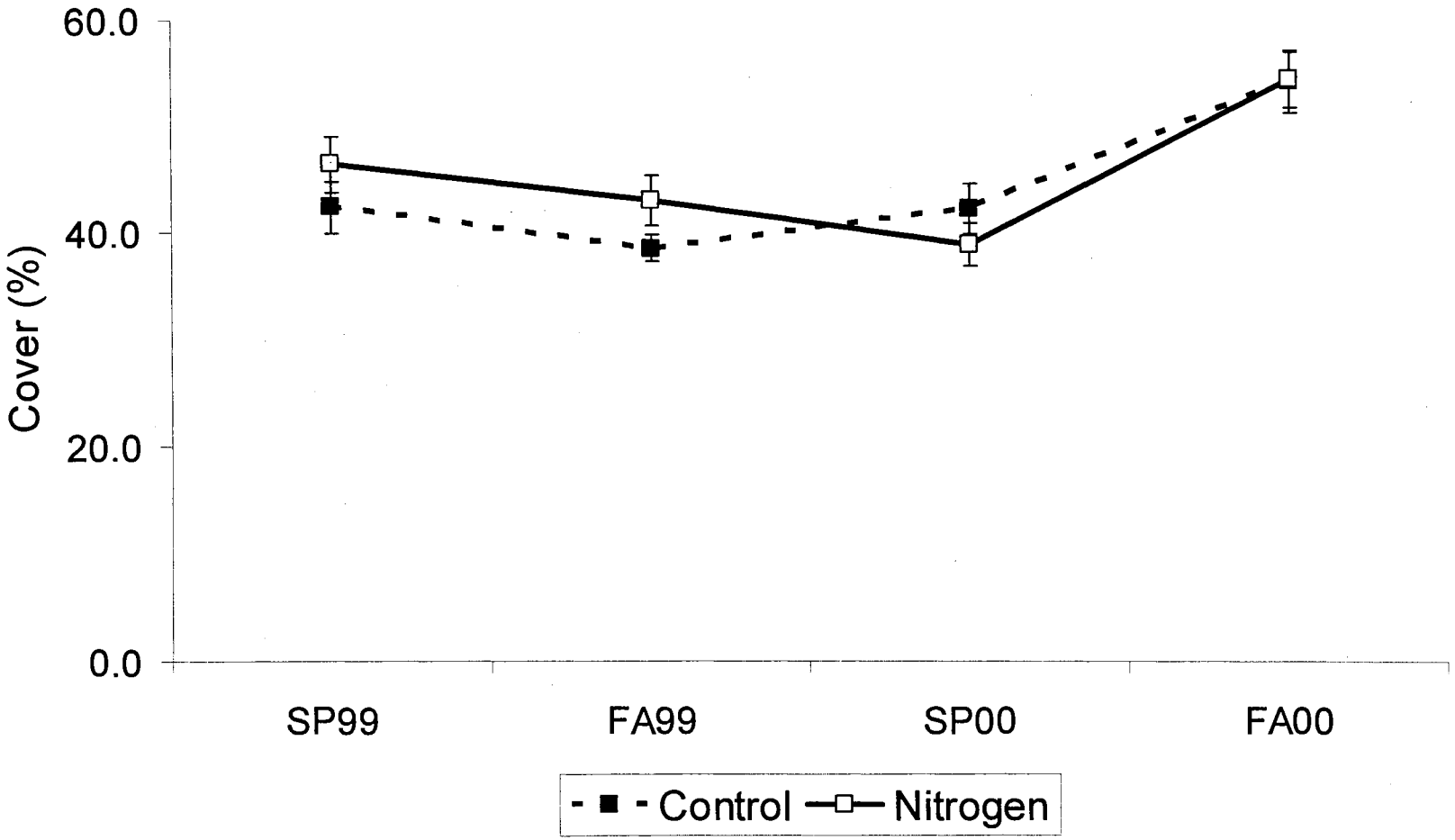
Figure I.4. Estimates and standard errors of minimum number known alive (MKNA) for hispid cotton rats (*Sigmodon hispidus*) across a landscape manipulated with nitrogen and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Each treatment comprised 4 replicate plots.

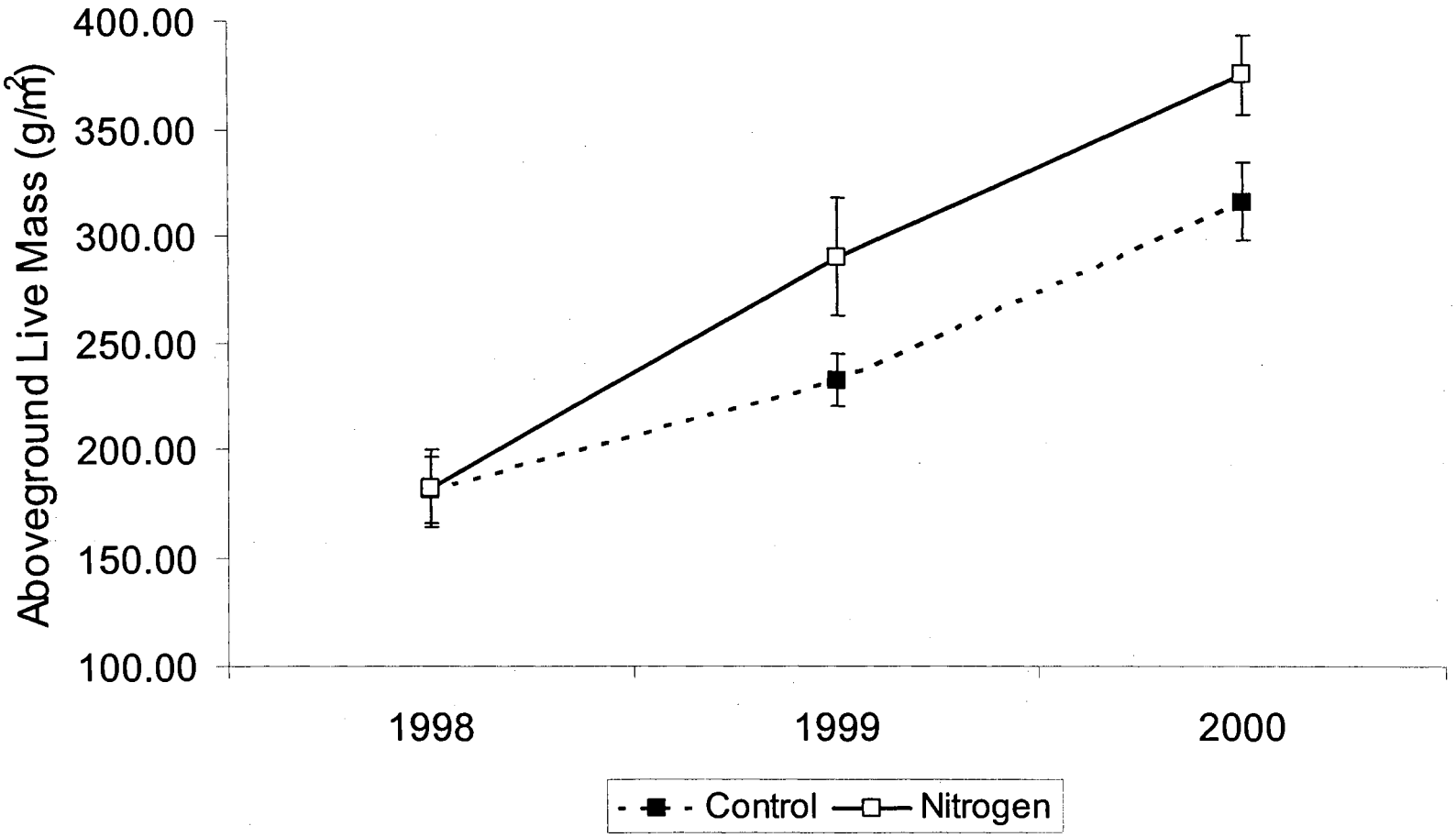
Figure I.5. Estimates and standard errors of juveniles/female for cotton rats (*Sigmodon hispidus*) across a landscape manipulated with nitrogen and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Each treatment comprised 4 replicate plots.

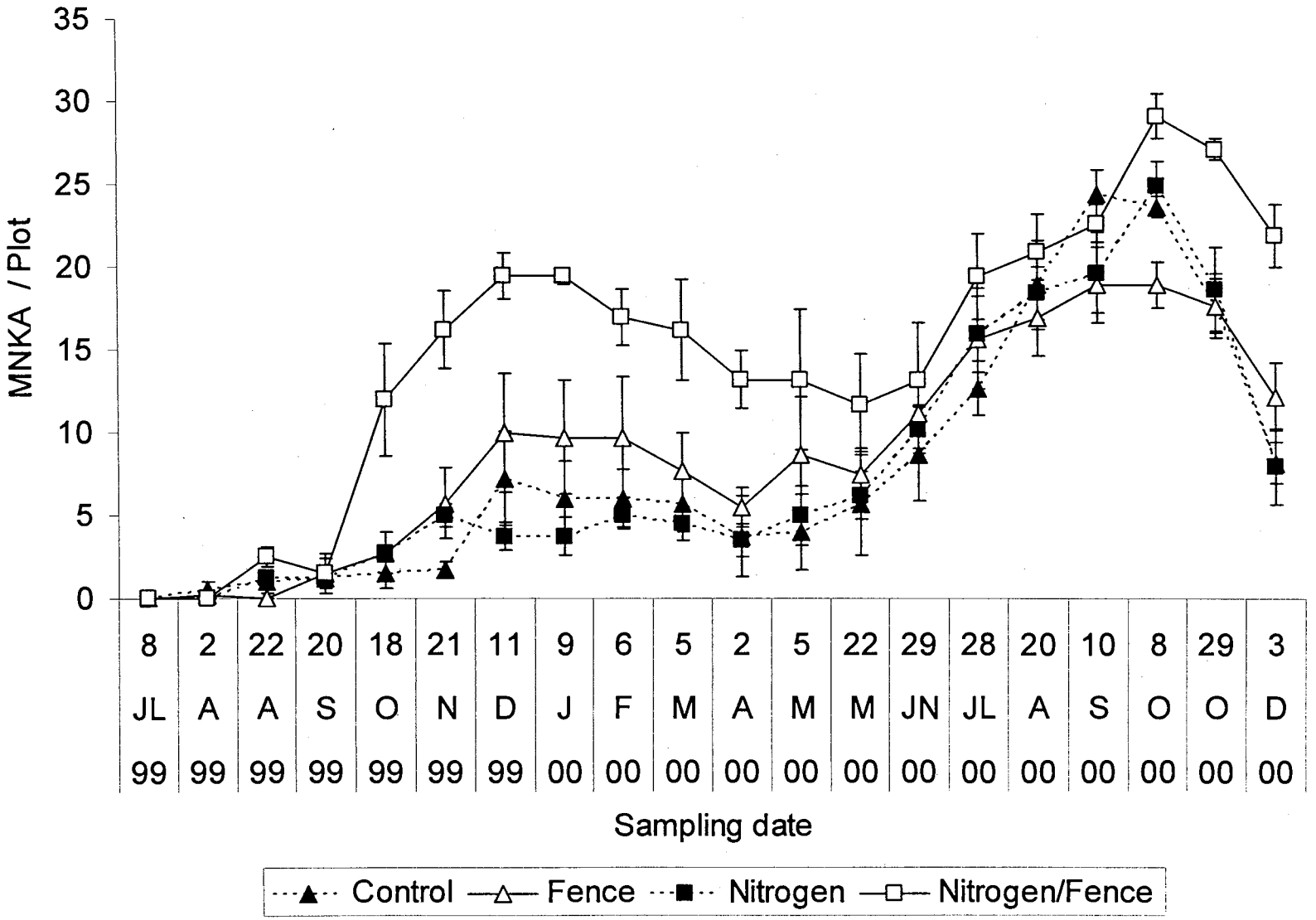
Figure I.6. Model-averaged survival estimates and standard errors of a) male and b) female hispid cotton rats (*Sigmodon hispidus*) on fenced and unfenced plots at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. We adjusted survival estimates to a 30-day interval. We used trapping data to determine breeding and nonbreeding seasons and defined an interval between trapping periods as breeding season if ≥10% of females were in reproductive condition (i.e., pregnant, lactating, or open vagina) during the latter trapping period (Breed 1 = 2 August 1999 – 21 November 1999; Non 1 = 22 November 1999 – 6 February 2000; Breed 2 = 7 February 2000 – 10 September 2000; Non 2 = 11 September 2000 – 3 December 2000).

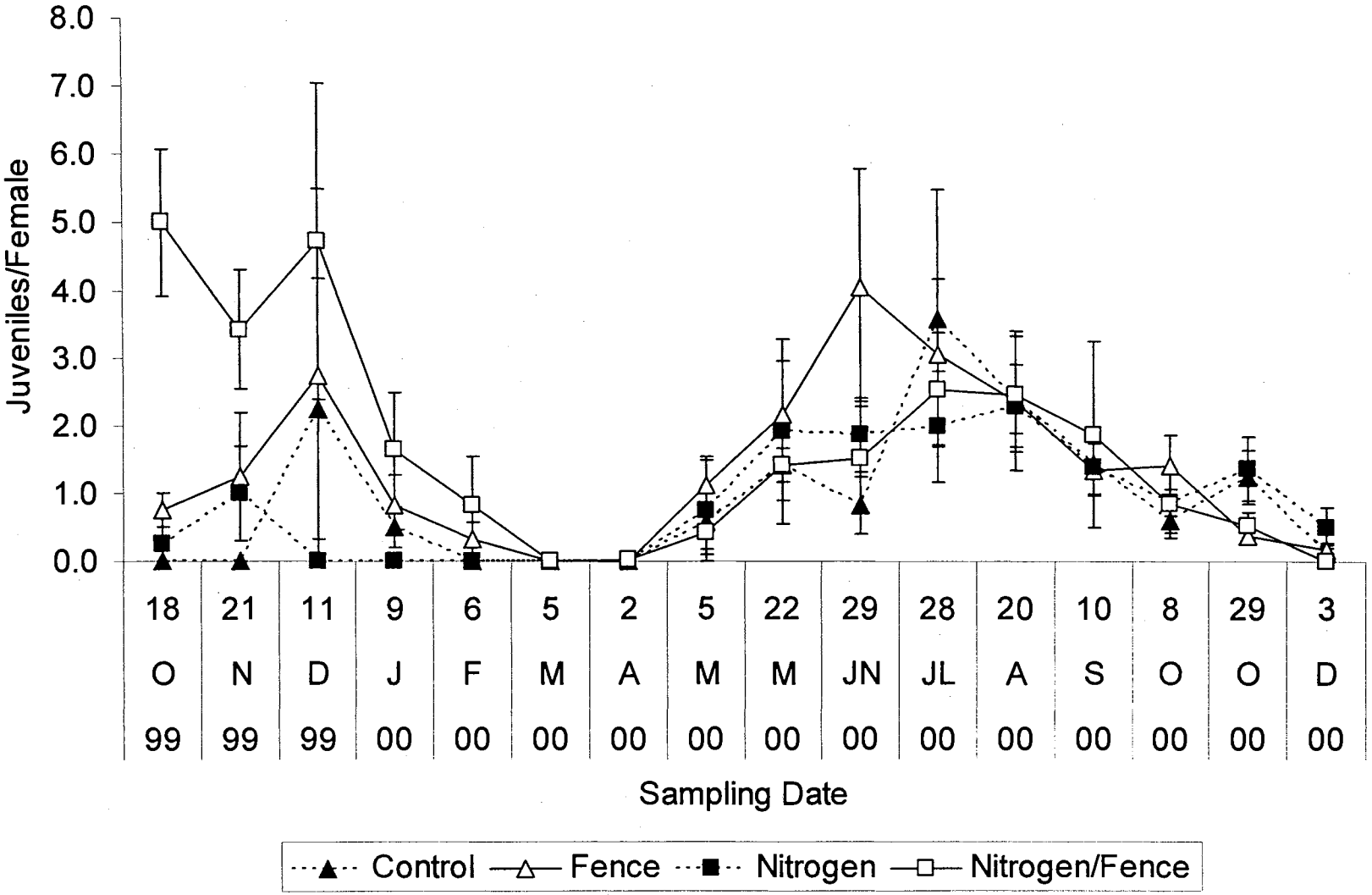
Figure I.7. Model-averaged transition probabilities of male hispid cotton rats (*Sigmodon hispidus*) across a landscape manipulated with nitrogen amendments and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999-2000. The larger the arrow between each treatment-to-treatment combination, the greater the probability of movement toward a particular strata. We adjusted transition probabilities to a 30-day interval.


5m
N
1
4
7
10
13
5m
2
5
8
11
14
3
6
9
12
15
16
40m
Nitrate Addition
Yes
No
Fenced
Yes
No

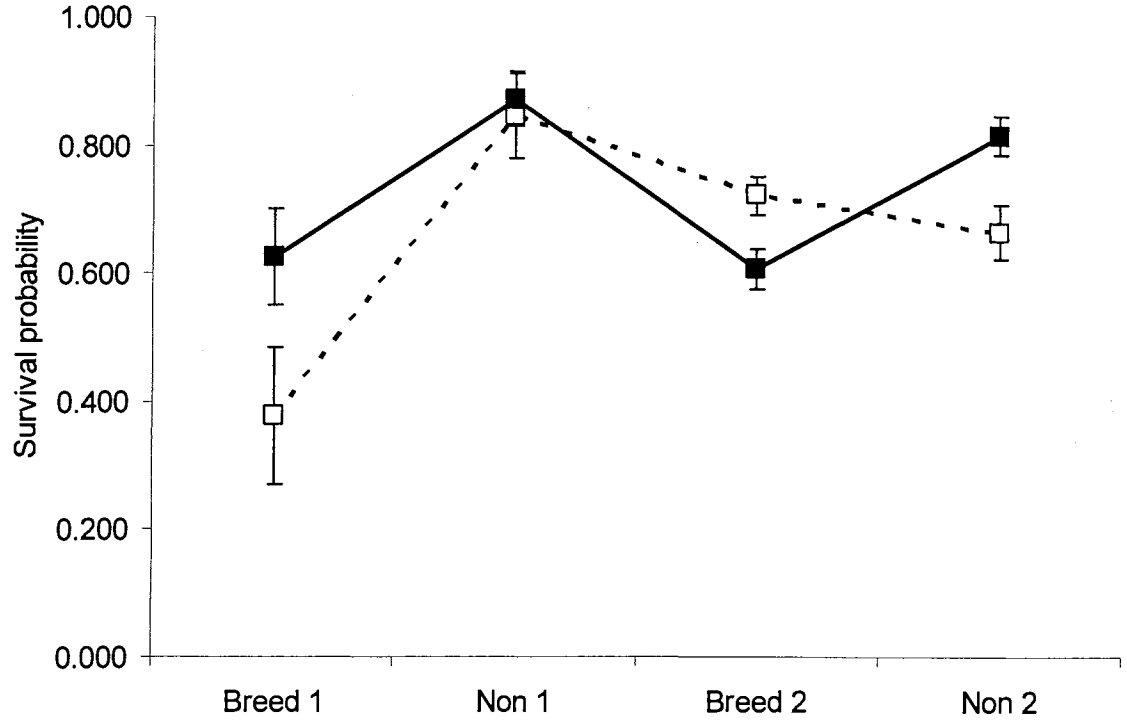

60.0
40.0
20.0
0.0
Cover (%)
SP99
FA99
SP00
FA00
Control
Nitrogen


400.00
350.00
300.00
250.00
200.00
150.00
100.00
Aboveground Live Mass (g/m2)
1998
1999
2000
Control
Nitrogen

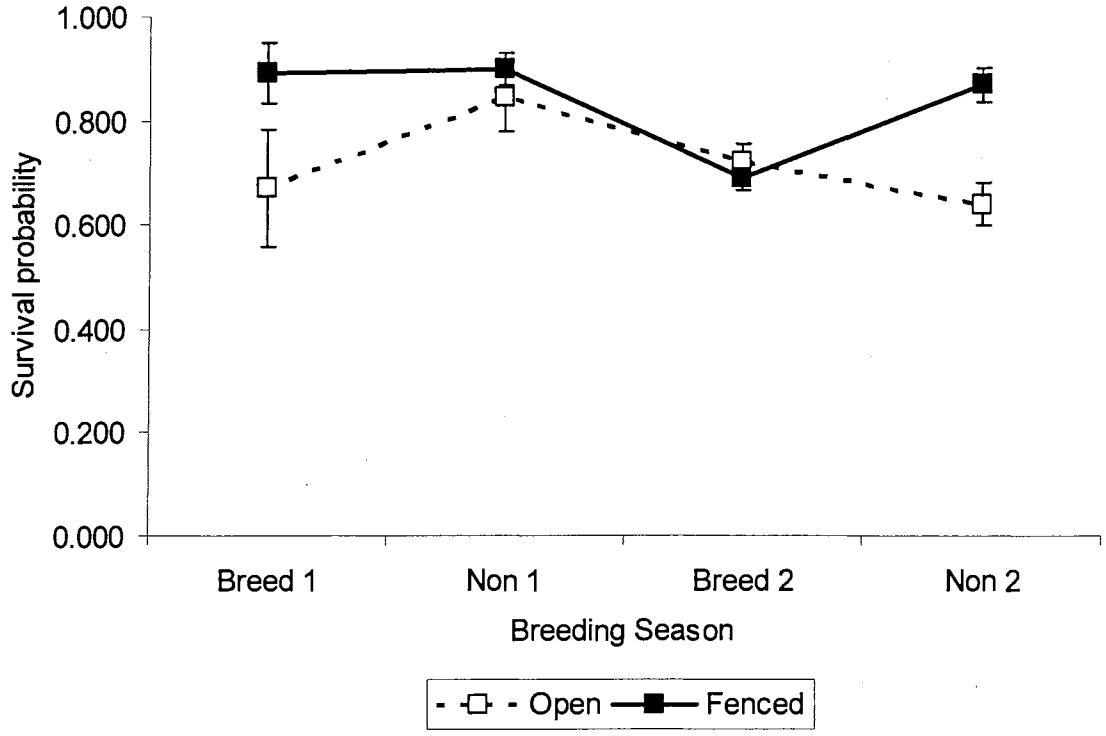

35
30
25
20
15
10
5
0
MNKA / Plot
8 JL 99
2 A 99
22 A 99
20 S 99
18 O 99
21 N 99
11 D 99
9 J 00
6 F 00
5 M 00
2 A 00
5 M 00
22 M 00
29 JN 00
28 JL 00
20 A 00
10 S 00
8 O 00
29 O 00
3 D 00
Sampling date
Control
Fence
Nitrogen
Nitrogen/Fence

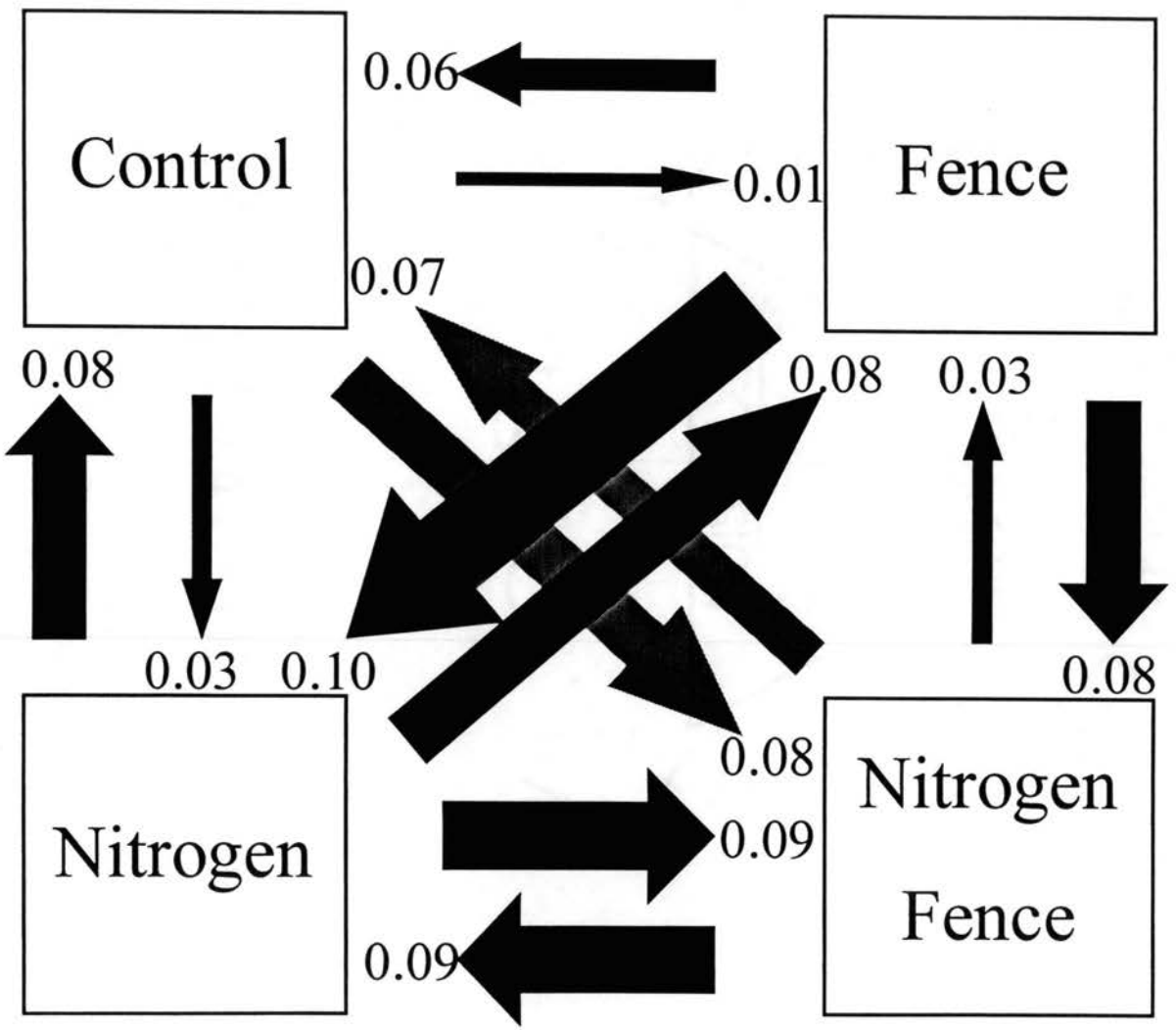

8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Juveniles/Female
18 O 99
21 N 99
11 D 99
9 J 00
6 F 00
5 M 00
2 A 00
5 M 00
22 M 00
29 JN 00
28 JL 00
20 A 00
10 S 00
8 O 00
29 O 00
3 D 00
Sampling Date
Control
Fence
Nitrogen
Nitrogen/Fence

a)


1.000
0.800
0.600
0.400
0.200
0.000
Survival probability
Breed 1
Non 1
Breed 2
Non 2

b)


1.000
0.800
0.600
0.400
0.200
0.000
Survival probability
Breed 1
Non 1
Breed 2
Non 2
Breeding Season
Open
Fenced


Control
Fence
Nitrogen
Nitrogen
Fence
0.06
0.01
0.07
0.08
0.08
0.03
0.03
0.10
0.08
0.08
0.09
0.09

CHAPTER II

POPULATION DYNAMICS OF HARVEST MICE (*REITHRODONTOMYS FULVESCENS* AND *REITHRODONTOMYS MONTANUS*) ACROSS A NITROGEN-AMENDED LANDSCAPE

Abstract: Population dynamics of some small-mammal species appear to be regulated by plant-community structure, vegetation cover, plant diversity, and food quality. Thus, changes in the plant community associated with nitrogen amendments would likely affect dynamics and structure of small-mammal populations. We conducted a mark-recapture experiment to examine population dynamics of the fulvous harvest mouse (*Reithrodontomys fulvescens*) and plains harvest mouse (*Reithrodontomys montanus*) in response to low-level nitrogen amendments (16.4 kg N/ha/yr) in an old-field grassland. The experimental design consisted of 16, 0.16-ha plots with 4 replicates of each treatment combination (fenced, nitrogen amendment; unfenced, nitrogen amendment; fenced, control; unfenced, control). We predicted that densities, survival, and transition probabilities would be greater for both species on nitrogen-amended plots because of greater aboveground biomass and cover. Population densities of *R. montanus* tended to be highest on nitrogen plots, but lowest on nitrogen-fenced plots during winter 1999–2000. This was opposite the pattern observed for hispid cotton rats (*Sigmodon hispidus*; Clark et al. *in review*) and may represent interspecific interaction between *R. montanus* and cotton rats. Survival of *R. montanus* did not exhibit any distinct patterns over time except for the non-breeding season in 2000, when survival was greater on fenced plots, regardless of nitrogen amendments. We observed no distinct patterns in survival or density of *R. fulvescens* with regard to treatments. Likewise, transition probabilities for both species did not vary across treatments. As low-level nitrogen

amendments continue to be applied, we predict survival and densities of *R. montanus* and *R. fulvescens* on control plots, especially fenced plots with no nitrogen amendment, will eventually exceed those on nitrogen-amended plots as a result of higher plant species diversity, food availability, and better quality cover; however, we postulate that the distribution of harvest mice, especially *R. montanus*, may be affected more by indirect effects (i.e., avoidance of areas with high densities of cotton rats) from nitrogen amendments.

Introduction

Future atmospheric deposition of nitrogen from anthropogenic activities is expected to increase as the global human population and reliance upon fossil fuels increases (Galloway et al. 1994, U.S. Environmental Protection Agency 1995). Thus, the amount of unretained nitrogen cycling through ecosystems is likely to increase and reasonably can be expected to cause future environmental problems. These biogeochemical alterations of the nitrogen cycle can dramatically change dominance hierarchies among species and decrease overall biodiversity of ecosystems by altering species composition, species diversity, and structure of food webs (Vitousek et al. 1997*a*, Vitousek et al. 1997*b*).

Nitrogen enrichment causes qualitative and quantitative changes in grassland vegetation. These changes result in an increase in biomass and decrease in plant species diversity (Grant et al. 1977, Tilman 1987, Carson and Barrett 1988, Hall et al. 1991, Tilman 1996, Wilson and Tilman 2002, Clark et al. *in review*). For example, after 9 years of annual nutrient enrichment, Carson and Barrett (1988) reported 3 contrasting

types of old-field communities with regard to structure and composition and a decrease in species diversity on nutrient-rich plots. Furthermore, long-term nitrogen amendments in artificially constructed plant communities dominated by C_4 prairie grasses resulted in nitrogen-mediated shifts to C_3 nonnative grasses (Wedin and Tilman 1996).

Population dynamics of some small-mammal species appear to be regulated by plant-community structure, vegetative cover, plant diversity, and food quality (Hall et al. 1991). Thus, plant community changes associated with nitrogen amendments would likely impact dynamics and structure of small-mammal populations. Hall et al. (1991) reported changes in meadow vole (*Microtus pennsylvanicus*) populations resulting from changes in plant community structure, cover, and diversity after supplementation of nitrogen. Although aboveground biomass was higher on nitrogen-amended plots, non-amended plots had higher plant diversity and quality of cover, resulting in positive population growth rates and higher population densities, rates of recruitment, and survivorship for voles (Hall et al. 1991). Similarly, Anderson and Barrett (1982) reported increased aboveground biomass and decreased plant species diversity on nutrient-amended sites, resulting in decreased densities and survivorship of meadow voles.

Populations of fulvous harvest mice (*Reithrodontomys fulvescens*) and plains harvest mice (*R. montanus*) are sympatric and common in old-field habitats of central Oklahoma (Goertz 1963). Both species rely heavily on invertebrates and seeds (Brown 1946, Gaertner 1968, Kincaid and Cameron 1982), and Goertz (1963) reported a tendency toward habitat segregation with *R. fulvescens* using areas of heavier grassy cover than *R. montanus*. Although dynamics of *R. fulvescens* and *R. montanus* have not been examined with respect to nitrogen amendments, increases in aboveground biomass

and cover resulting from nitrogen amendments would likely influence population distribution and dynamics. In Texas, Spencer and Cameron (1985) reported higher use of unmowed compared to mowed habitats by *R. fulvescens* and attributed this difference to removal of the vertical component (i.e., shrub layer) of habitat structure. Similarly, densities of *R. fulvescens* were higher in unmowed patches compared to mowed patches (Spencer and Cameron 1985).

Our objective was to examine how population dynamics of *R. fulvescens* and *R. montanus* responded to an old-field landscape altered by low-level nitrogen amendments (16.4 kg N/ha/yr) and exclosure fencing (i.e., constraints on herbivory). Because changes in plant species richness and abundance are not as speedily observed as changes in aboveground biomass (Tilman 1987, 1988; Wedin and Tilman 1996), we did not expect to observe a decrease in plant species diversity on nitrogen-amended plots given the duration of our study (<2 yr). However, we did expect increases in aboveground biomass and cover on nitrogen-amended plots. Thus, we predicted that densities, survival rates, and transition probabilities of both species of harvest mice would be higher on nitrogen-amended plots as a result of increased aboveground plant biomass and cover.

Study Area

The field research was conducted at the Environmental Protection Agency's Center for Subsurface and Ecological Assessment Research (CSEAR) near Garr Corner, Pontotoc County, Oklahoma. The study area was an old-field site composed of 16 square 0.16-ha experimental plots surrounded and separated from adjacent plots by a 5-m uninhabitable mowed strip (total area = 3.45 ha; Figure II.1). The area had not been cultivated since 1950 but was heavily grazed during the last half-century. Dominant

vegetation consisted of early to mid-successional grasses and forbs, including old-field threeawn (*Aristida oligantha*), broomsedge bluestem (*Andropogon virginicus*), western ragweed (*Ambrosia psilostachya*), and heath aster (*Aster ericodes*). Preliminary sampling of small mammals at CSEAR identified existing populations of mice (i.e., *Reithrodontomys* spp. and *Peromyscus* spp.) and least shrews (*Cryptotis parva*), but hispid cotton rats (*Sigmodon hispidus*) were absent on our experimental plots before sampling for our study (E. E. Jorgensen, unpublished data). This initial absence of cotton rats was not unexpected, because Phillips (1936) had previously reported an absence of hispid cotton rats in overgrazed pastures in central Oklahoma.

The experimental design consisted of 2 treatments (exclosure fencing and nitrogen amendment) randomly applied to the study plots in a 2 × 2 factorial with 4 replicates per treatment (fenced, nitrogen amendment; fenced, control; unfenced, nitrogen amendment; unfenced, control). Fenced plots were surrounded by a 2-m high, 2.5-cm chain-link fence that allowed free movement of small mammals between plots but restricted access by terrestrial predators and larger mammalian herbivores (e.g., lagomorphs, artiodactyls). To ensure free movement between plots, 7.5-cm triangular holes were cut at ground level and spaced at 2-m intervals around fenced plots. Beginning in February 1999, we added nitrogen fertilizer (34% ammonium nitrate) to nitrogen-amended plots at a rate of 3.1, 5.1, 4.1, and 4.1 kg N/ha in February, May, August, and November, respectively. Nitrogen amendment rates corresponded to seasonal rainfall proportions for central Oklahoma. Oklahoma receives approximately 10 kg/ha/yr of nitrate via atmospheric deposition (National Atmospheric Deposition Program (NRSP-3)/National Trends Network 2002). Nitrogen amendments of 16.4 kg

N/ha/yr were chosen for the study because rates of atmospheric deposition of nitrogen in excess of this occur in industrialized regions of the globe and are projected to increase for the foreseeable future (Brimblecombe and Stedman 1982, Galloway et al. 1994, U.S. Environmental Protection Agency 1995, Vitousek et al. 1997*b*). Clark et al. (*in review*) sampled aboveground live-mass and canopy cover throughout the current study and reported differences in aboveground live-mass between nitrogen-amended and non-amended plots following initiation of nitrogen applications.

Methods

Small-mammal sampling— We sampled small mammals with Sherman live traps (7.6 × 8.9 × 22.9 cm) for 3 consecutive days at 3–5-week intervals from July 1999 to December 2000. Each plot consisted of 25 traps systematically spaced at 7-m intervals; thus, we had a potential of 1,200 trap nights/sampling period. Traps were set each afternoon, baited with rolled oats, checked between 0600 and 1200 hours, and provided with cotton for warmth during cold weather. We released captured animals immediately after marking with a unique number via toe clipping. We recorded the trap station, species, mass, sex, and reproductive status (scrotal or non-scrotal for males; pregnant, lactating, and open or closed vagina for females) for each individual. Additionally, we recorded instances of accidentally sprung traps (i.e., sprung traps not resulting in capture).

We used minimum number known alive (MNKA; Krebs 1966) as an index to abundance for each plot at each sampling period. We made statistical comparisons of MNKA between treatment plots using 2-way analysis of variance with repeated measures

(PROC MIXED, SAS 2000). We fitted a multiple variance model using the Kenward-Roger approximation to calculate effective degrees of freedom (PROC MIXED, SAS 2000; Kenward and Roger 1997) and used least-squared means separation tests for all significant main effects. Because small mammals were colonizing experimental plots during the first few sampling periods and number of captures for all species was low, statistical comparisons of MNKA were limited to October 1999–December 2000 (i.e., 16 trapping periods). A significance level of $P \leq 0.05$ was used for all analyses.

Population modeling.— We used a multi-strata model (Hestbeck et al. 1991, Brownie et al. 1993) in Program MARK (White and Burnham 1999) to estimate apparent survival, capture probabilities, and transition probabilities of *R. montanus* and *R. fulvescens* across our study plots. Modeling for potential differences in survival between strata was our primary interest; thus, we examined models with varying strata effects (i.e., no treatments, nitrogen only, fence only, and combination of fence and nitrogen) for survival. Models were ranked using Akaike's Information Criterion (AICc) and were averaged to determine final parameter estimation using AICc weights (Burnham and Anderson 1998). Our global model (i.e., most parameterized) included strata effects for all parameters and time effects (i.e., breeding or non-breeding season) for survival and recapture probabilities, but did not include sex effects. We used trapping data for each species to determine breeding and nonbreeding seasons and defined an interval between trapping periods as breeding season if ≥10% of females were in reproductive condition (i.e., pregnant, lactating, or open vagina) during the latter trapping period.

For all models, we accounted for varying time intervals between sampling periods (3–5 weeks) by adjusting parameter estimates to a 30-day interval between periods. We

adjusted recapture probabilities for varying sampling effort among strata by accounting for sprung traps (i.e., traps that captured animals and those accidentally sprung) in all models. Lacking specific information on timing of trap-springing, we assumed that each trap was sprung halfway between trap-setting and trap-checking (Nelson and Clark 1973, Beauvais and Buskirk 1999). Thus, sampling effort was calculated for each stratum as:

$$\frac{\sum_i t_i - (s_i \times 0.5)}{b},$$

where t was the maximum number of potential trap nights within trapping period i (i.e., maximum = 300 trap nights / stratum), s was the number of sprung traps at trapping period i, and b was the maximum number of potential trap nights during a season (i.e., breeding or nonbreeding).

Examination of instantaneous sampling assumption.— Similar to the standard Jolly-Seber model, the multi-strata model assumes all samples are instantaneous (i.e., all mortality and movement occurs between, not within, sampling periods; Hestbeck et al. 1991); however, this assumption can never be strictly met (Hestbeck et al. 1991). We examined departures from this assumption by identifying all occasions when an individual harvest mouse was captured in multiple strata within a sampling period. The encounter histories in the original analysis for those individuals were coded by recording the stratum where each mouse was initially captured as the encounter for a given sampling period. To investigate violations of the instantaneous sampling assumption, we performed an additional analysis (Test-1) for each species of harvest mouse with the original candidate set of models to determine if model rankings, identified patterns, and estimates of survival from the original analysis were robust to departures from this

assumption. The Test-1 analyses were performed by changing the encounter history for each harvest mouse captured on multiple strata within a sampling period to the alternative stratum.

Results

Small-mammal sampling and modeling.— Between July 1999 and December 2000, we recorded 7,955 small-mammal captures in 20 sampling periods (i.e., 24,000 potential trap nights). *R. montanus* accounted for 1,229 captures of 308 individuals (males = 178, females = 130) and *R. fulvescens* accounted for 742 captures of 182 individuals (males = 87, females = 95). Cotton rats accounted for 5,468 captures of 982 individuals (males = 538, females = 444) and were the most abundant small-mammal species on our experimental plots. Abundance of *R. montanus* tended to be highest on nitrogen plots, but lowest on nitrogen-fenced plots during winter 1999–2000 (3-way interaction: nitrogen × fence × time, $F_{15, 171} = 2.22$, $P = 0.007$; Figure II.2). We observed no distinct patterns (i.e., significant effects) in relation to the treatment plots for MNKA estimates of *R. fulvescens* (Figure II.3).

The minimum AICc multi-strata model for *R. montanus* provided estimates of survival on fenced and unfenced plots with transition probabilities constant across strata (Table II.1). Survival probabilities exhibited no distinct pattern over time except for the non-breeding season in 2000 when survival was greater on fenced plots (Figure II.4*a*). The minimum AICc model for *R. fulvescens* included survival and transition probabilities constant across strata (Table II.2). Monthly survival estimates for *R. fulvescens* ranged from 0.70 (SE = 0.09; breeding season 1999) to 0.80 (SE = 0.04; non-breeding season

1999–2000; Figure II.5). Transition probabilities among strata from the minimum AICc model for *R. montanus* and *R. fulvescens* were 0.07 and 0.08, respectively.

Examination of instantaneous sampling assumption– Both species of harvest mice were captured on multiple strata within a sampling period; thus, we evaluated effects of violating the instantaneous sampling assumption by performing an additional analysis (Test-1) for each species with the original candidate set of models. The minimum AICc models and respective AICc weights for *R. fulvescens* were similar across analyses. Percent differences in estimates of survival for *R. fulvescens* between the original analysis and Test-1 in all other seasons were $\leq 5.2\%$, and patterns with respect to the treatments were the same. Thus, we assumed that our original analysis for *R. fulvescens* was robust to violations of the instantaneous sampling assumption.

The minimum AICc models and respective AICc weights for *R. montanus* varied across analyses. Unlike the original analysis, the Test-1 analysis did not support a fence effect (i.e., higher survival in fenced plots), and model-averaged estimates for the second non-breeding season (Non 2 = 11 September 2000–3 December 2000) varied across analyses (Table II.3; Figure II.4*b*). However, the percent differences in estimates of survival between the original analysis and Test-1 in all other seasons were ≤ 10 %, and patterns with respect to the treatments were the same. Thus, we assumed that our original analysis for *R. montanus* was robust to violations of the instantaneous sampling assumption, but caution that survival estimates for the second non-breeding season did not conclusively support a fence effect.

Discussion

Our predictions concerning changes in population parameters of harvest mice at CSEAR were based primarily on increased cover availability on nitrogen-amended plots. Although nitrogen amendments to old-field plots at CSEAR caused increases in total aboveground live mass (Clark et al. *in review*), we observed little evidence to support our predictions concerning changes in population dynamics of harvest mice and nitrogen amendments. During the current study, Clark et al. (*in review*) found that population dynamics of cotton rats at CSEAR were influenced more by the presence of fences than nitrogen amendments. Although densities tended to be higher on nitrogen-fenced plots compared to other plots, survival and reproductive success of cotton rats were higher on fenced plots, regardless of nitrogen amendments (Clark et al. *in review*).

Evidence supporting our prediction of higher abundances of *R. montanus* on nitrogen-amended plots was limited. Abundances of *R. montanus* tended to be highest on nitrogen plots but lowest on nitrogen-fenced plots (Figure II.3). The opposite pattern was observed for cotton rats (Clark et al. *in review*) and may be attributed to negative interference and avoidance of cotton rats by *R. montanus*. Post-hoc exploration of the data identified a negative relationship between abundances of cotton rats and *R. montanus* (Figure II.6). For example, plots with high densities of cotton rats tended to have decreased densities of *R. montanus* compared to plots with lower densities of cotton rats (Figure II.6).

Avoidance of contact with cotton rats by sympatric species has been reported (Terman 1974, Glass and Slade 1980). Although interactions between cotton rats and *R. montanus* are unknown, sympatric populations of *R. fulvescens* and cotton rats are known

to coexist with little evidence of competition (Cameron 1977, Joule and Cameron 1980). Cameron and Kincaid (1982) reported larger proportions of captures in aboveground traps for *R. fulvescens* in the presence of cotton rats and suggested increased use in aboveground vegetation strata may be a response to avoid encounters with cotton rats. Although *R. fulvescens* and other species in the genus *Reithrodontomys* are scansorial (Rosenzweig et al. 1975, Meserve 1976), it is unclear whether *R. montanus* is a prolific climber. If not, *R. montanus* may not be as efficient as *R. fulvescens* at avoiding cotton rats, thus, forcing *R. montanus* to occupy habitats where cotton rats are less dense. Despite a clear negative relationship between cotton rats and *R. montanus* across our study area (Figure II.6), we caution that our inference was based on post-hoc exploration and should be investigated in future experiments with sympatric populations of these species.

Survival of *R. montanus* did not vary across treatments except during the second non-breeding season when survival appeared to be higher on fenced plots (Figure II.4*a*). Clark et al. (*in review*) reported a similar "fence-effect" for cotton rats and suggested decreased predation on fenced plots. However, we caveat against drawing strong inference from the apparent fence effect for *R. montanus*, because results of the analysis to test violations of the instantaneous sampling assumption (i.e., Figure II.4*b*) and original analysis were conflicting. Our Test-1 analysis represented the most conservative (i.e., worst case scenario) approach to addressing violations of the instantaneous assumption. Although results were conflicting between analyses in relation to the fence treatment, results from the 2 analyses were consistent with respect to nitrogen amendments and did not support a nitrogen effect; thus, we considered inferences with

respect to nitrogen-amendments to be robust to violations of the instantaneous sampling assumption.

We observed no distinct relationships between population characteristics of *R. fulvescens* and experimental plots; thus, it appears that population characteristics of *R. fulvescens* were not influenced by changes in habitat following nitrogen amendments or presence of fences. Monthly survival estimates of *R. fulvescens* at CSEAR were higher than estimates of survival from populations in coastal Texas (ca. 0.25–0.43; Spencer and Cameron 1985) and Mexico (0.5–0.75 / 3weeks; Petersen 1978). Abundances of *R. fulvescens* remained low throughout our study (Figure II.3) compared to *R. montanus* (Figure II.2) and cotton rats (Clark et al. *in review*); however, average monthly density across the study area for *R. fulvescens* (9.5/ha) was comparable to population estimates from other studies (peaks of 11/ha and 28/ha, Cameron 1977; maximum of 18.1/ha, Spencer and Cameron 1985; 5.8/ha, Packard 1968). Additionally, we observed a bimodal density pattern with peaks in summer and winter as reported in other studies (Packard 1968, Cameron 1977, Spencer and Cameron 1985).

Transition probabilities for *R. montanus* and *R. fulvescens* displayed no clear trends and did not support our predictions related to movements toward nitrogen-amended plots. We note that transition probabilities were calculated for strata-to-strata movements (i.e., not plot-to-plot) and held constant across time. Because movement rates may vary temporally (e.g., increased movements during breeding activities), a more detailed examination of movements incorporating seasonal effects and plot-to-plot movements could provide important information pertaining to dispersal and distribution

across our experimental plots. Furthermore, these estimates would provide possible explanations for temporal differences in survival of harvest mice at CSEAR.

As with plants (Tilman 1987, 1988; Wedin and Tilman 1996), it is possible that small-mammal communities will decrease in species richness and become dominated by a few successful competitors that respond to increased nitrogen availability. Interactions between hispid cotton rats, the dominant species of small mammal at CSEAR, and other species of small mammals may influence population dynamics across our experimental plots. As low-level nitrogen amendments continue to be applied at CSEAR, Clark et al. (*in review*) predicted nitrogen-mediated shifts in the distribution of cotton rats from nitrogen-amended to non-amended plots as a result of the cumulative effect of nitrogen amendments on habitat quality (i.e., decreased species diversity, food availability, and quality of cover on nitrogen-amended plots). Likewise, we predict nitrogen-mediated shifts in the distribution of harvest mice; however, we postulate that the distribution of harvest mice, especially *R. montanus*, may be affected more by indirect effects (i.e., avoidance of areas with high densities of cotton rats) than direct effects of nitrogen amendments.

Acknowledgments

This project was funded by the United States Environmental Protection Agency (EPA) under Interagency Agreement DW14938554-01-0 with the United States Geological Survey's (USGS) Biological Resources Division and administered by the Oklahoma Cooperative Fish and Wildlife Research Unit (Oklahoma State University, Oklahoma Department of Wildlife Conservation, USGS's Biological Resources Division,

and Wildlife Management Institute cooperating). The views expressed in this manuscript are those of the authors and do not necessarily reflect the views and policies of EPA or USGS. Additional funding was provided through a fellowship from the Environmental Institute at Oklahoma State University. We thank M. Payton for statistical advice. We appreciate the field assistance of J. Lindsey, P. Hanson, L. Levesque, M. Bahm, J. Bahm, M. Leslie, and K. Brazil and logistical assistance of J. Gray, H. Murray, and S. Lyon. The contributions of the late R. L. Lochmiller were important to the genesis of this project.

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Table II.1. Model parameters and AICc values for multi-strata models examining survival (*S*) and transition probabilities (*PSI*) of plains harvest mice (*Reithrodontomys montanus*) across a landscape manipulated with nitrogen amendments and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Capture probabilities (*p*) varied by breeding season across all strata in all models.

Model	ΔAICc	AICc weights	Number of parameters	Deviance
{*S*(breeding)FENCE *p*(breeding)ALL *PSI* NO STRATA}[a]	0.00	0.67	23	1,601.236
{*S*(breeding)NO STRATA *p*(breeding)ALL *PSI* NO STRATA}[b]	2.60	0.18	22	1,605.958
{*S*(breeding)NO STRATA *p*(breeding)ALL *PSI* ALL}[c]	4.53	0.07	31	1,588.571
{*S*(breeding)NITRO *p*(breeding)ALL *PSI* ALL}[d]	5.15	0.05	34	1,582.644

[a] Survival varies by breeding season for fenced and unfenced plots (i.e., no nitrogen effect), and transition probabilities are constant across all strata.

[b] Survival is constant across all strata and varies by breeding season, and transition probabilities are constant across all strata.

Table II.1. (Continued)

[c] Survival is constant across all strata and varies by breeding season, and transition probabilities vary across all strata.

[d] Survival varies by breeding season for nitrogen-amended and non-amended plots (i.e., no fence effect), and transition probabilities vary across all strata.

Table II.2. Model parameters and AICc values for multi-strata models examining survival (*S*) and transition probabilities (*PSI*) of fulvous harvest mice (*Reithrodontomys fulvescens*) across a landscape manipulated with nitrogen amendments and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Capture probabilities (*p*) varied by breeding season across all strata in all models.

Model	ΔAICc	AICc weights	Number of parameters	Deviance
{*S*(breeding)NO STRATA *p*(breeding)ALL *PSI* NO STRATA}[a]	0.00	0.88	19	1,395.286
{*S*(breeding)FENCE *p*(breeding)ALL *PSI* NO STRATA}[b]	4.36	0.10	25	1,386.416
{*S*(breeding)NITRO *p*(breeding)ALL *PSI* NO STRATA}[c]	7.89	0.02	23	1,394.395
{*S*(breeding)ALL *p*(breeding)ALL *PSI* NO STRATA}[d]	13.16	0.00	31	1,381.636

[a] Survival is constant across all strata and varies by breeding season, and transition probabilities are constant across all strata.

[b] Survival varies by breeding season for fenced and unfenced plots (i.e., no nitrogen effect), and transition probabilities are constant across all strata.

Table II.2. (Continued)

[c] Survival varies by breeding season for nitrogen-amended and non-amended plots (i.e., no fence effect), and transition probabilities are constant across all strata.

[d] Survival varies by breeding season across all strata, and transition probabilities are constant across all strata.

Table II.3. Model-averaged survival estimates from the original analysis and analysis (Test-1) examining violations to the instantaneous sampling assumption for *Reithrodontomys montanus* across experimental plots during the second non-breeding season (Non 2 = 11 September 2000–3 December 2000) at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma.

	Survival estimate		
Strata	Original	Test-1	% difference between Original vs. Test-1
Control	0.528	0.674	27.7
Fenced	0.890	0.749	18.8
Nitrogen	0.507	0.652	28.6
Fenced Nitrogen	0.864	0.736	17.4

Figure II.1. Layout of nitrogen and exclosure treatments for experimental plots at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000.

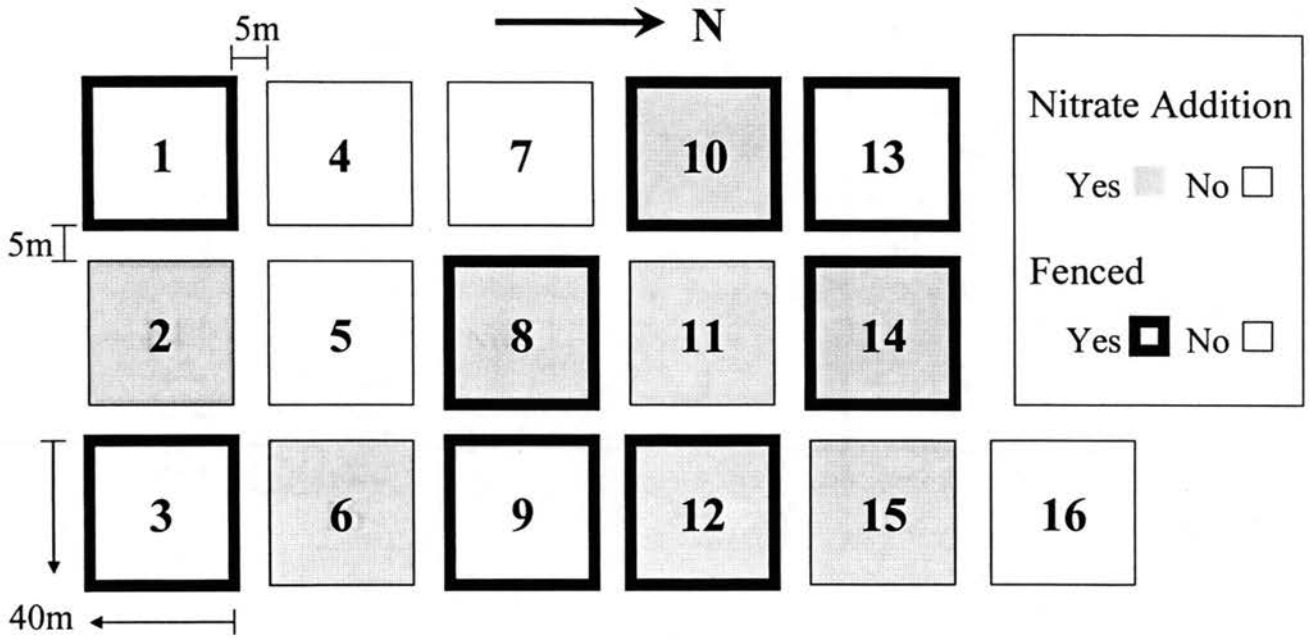
Figure II.2. Estimates and standard errors (±1 SE) of minimum number known alive (MKNA) for plains harvest mice (*Reithrodontomys montanus*) across a landscape manipulated with nitrogen and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Each treatment comprised 4 replicate plots.

Figure II.3. Estimates of minimum number known alive (MKNA) for fulvous harvest mice (*Reithrodontomys fulvescens*) across a landscape manipulated with nitrogen and exclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Each treatment comprised 4 replicate plots.

Figure II.4. Model-averaged survival estimates and standard errors (±1 SE) from the a) original and b) Test-1 analyses for the plains harvest mouse (*Reithrodontomys montanus*) across experimental plots at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Breeding and non-breeding seasons were determined from reproductive data (Breed 1 = 7 July 1999–11 December 1999, Non 1 = 12 December 1999–6 February 2000, Breed 2 = 7 February 2000–10 September 2000, Non 2 = 11 September 2000–3 December 2000).

Figure II.5. Model-averaged survival estimates and standard errors (±1 SE) for the fulvous harvest mouse (*Reithrodontomys fulvescens*) across experimental plots at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Breeding and non-breeding seasons were determined from reproductive data (Breed 1 = 7 July 1999–21 November 1999, Non 1 = 22 November 1999–6 February 2000, Breed 2 = 7 February 2000–8 October 2000, Non 2 = 9 October 2000–3 December 2000).

Figure II.6. Relationship of minimum number known alive (MNKA) for the plains harvest mouse (*Reithrodontomys montanus*) and hispid cotton rat (*Sigmodon hispidus*) across experimental plots at the Center for Subsurface Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Points represent the estimate of MKNA/plot for each species during each sampling period.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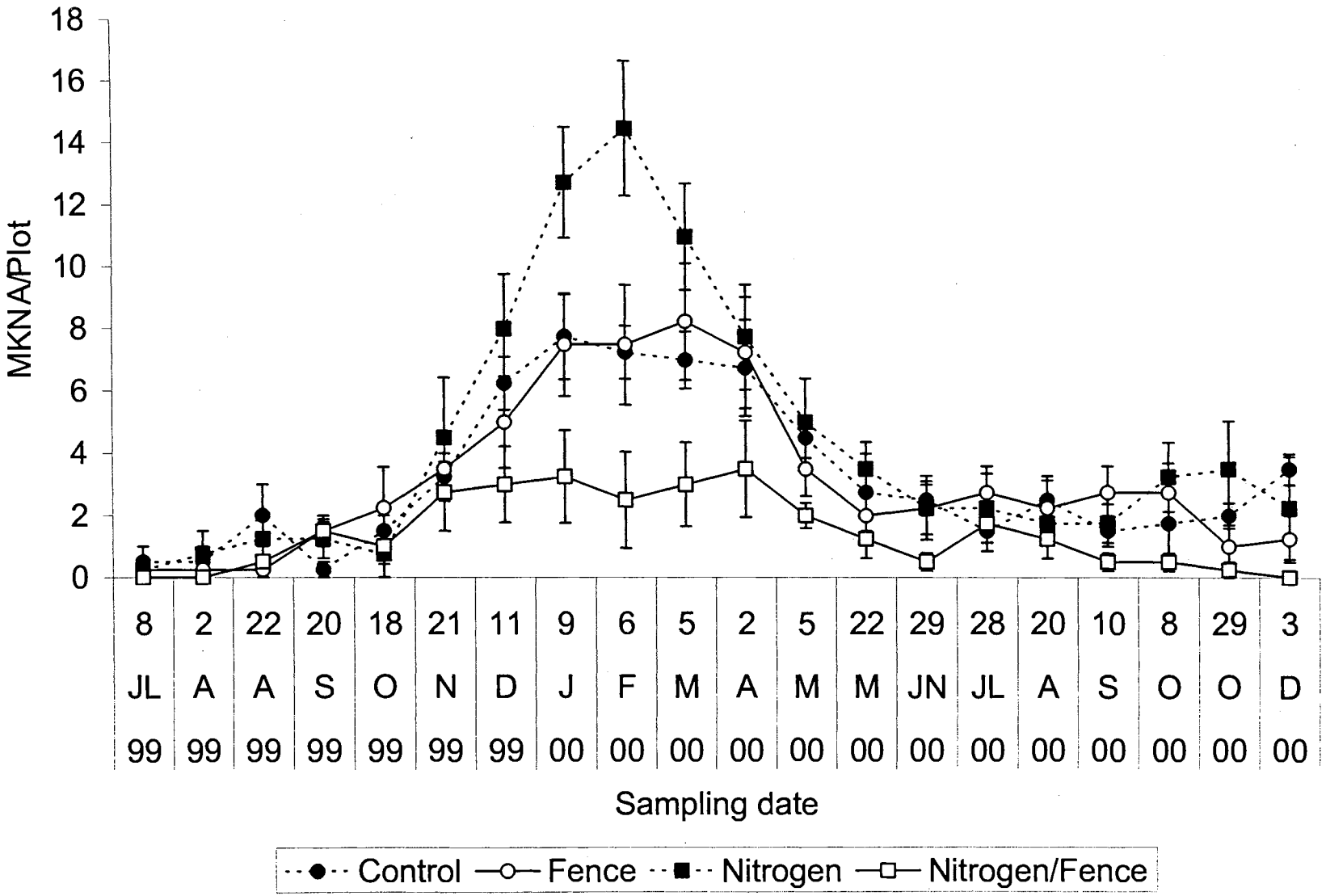
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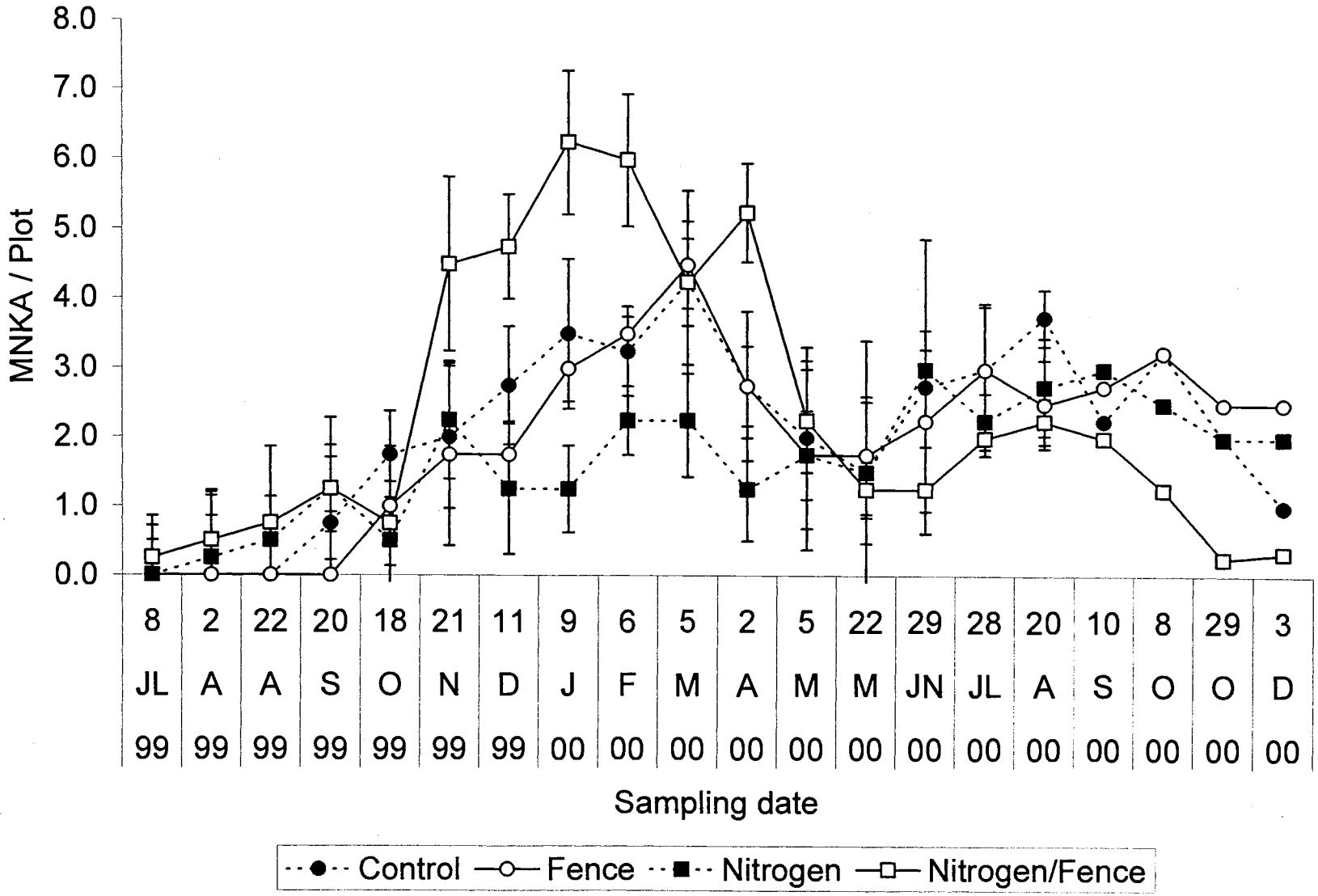
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21 NV 99
11 DC 99
9 JN 00
6 FB 00
5 MR 00
2 AP 00
5 MY 00
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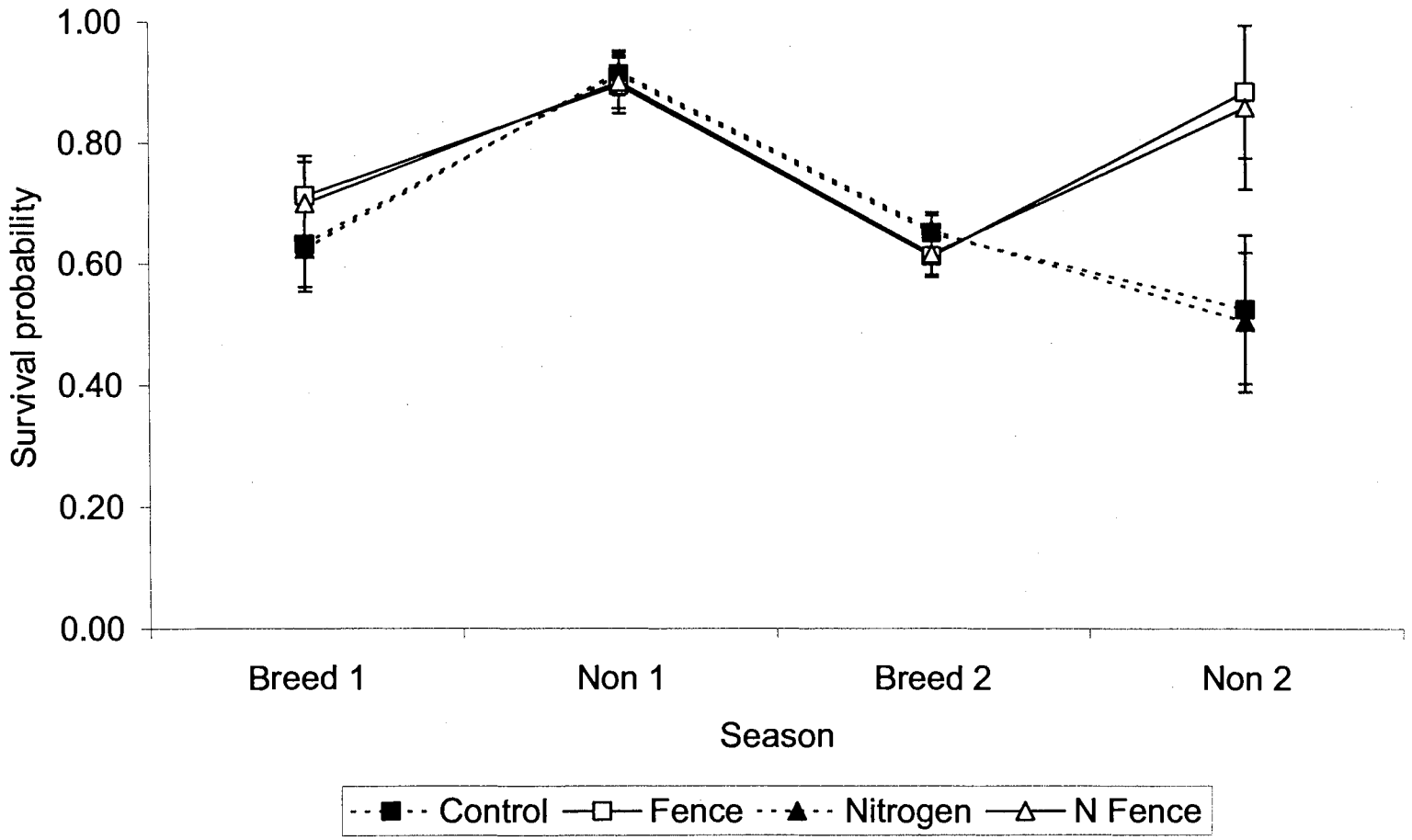
a)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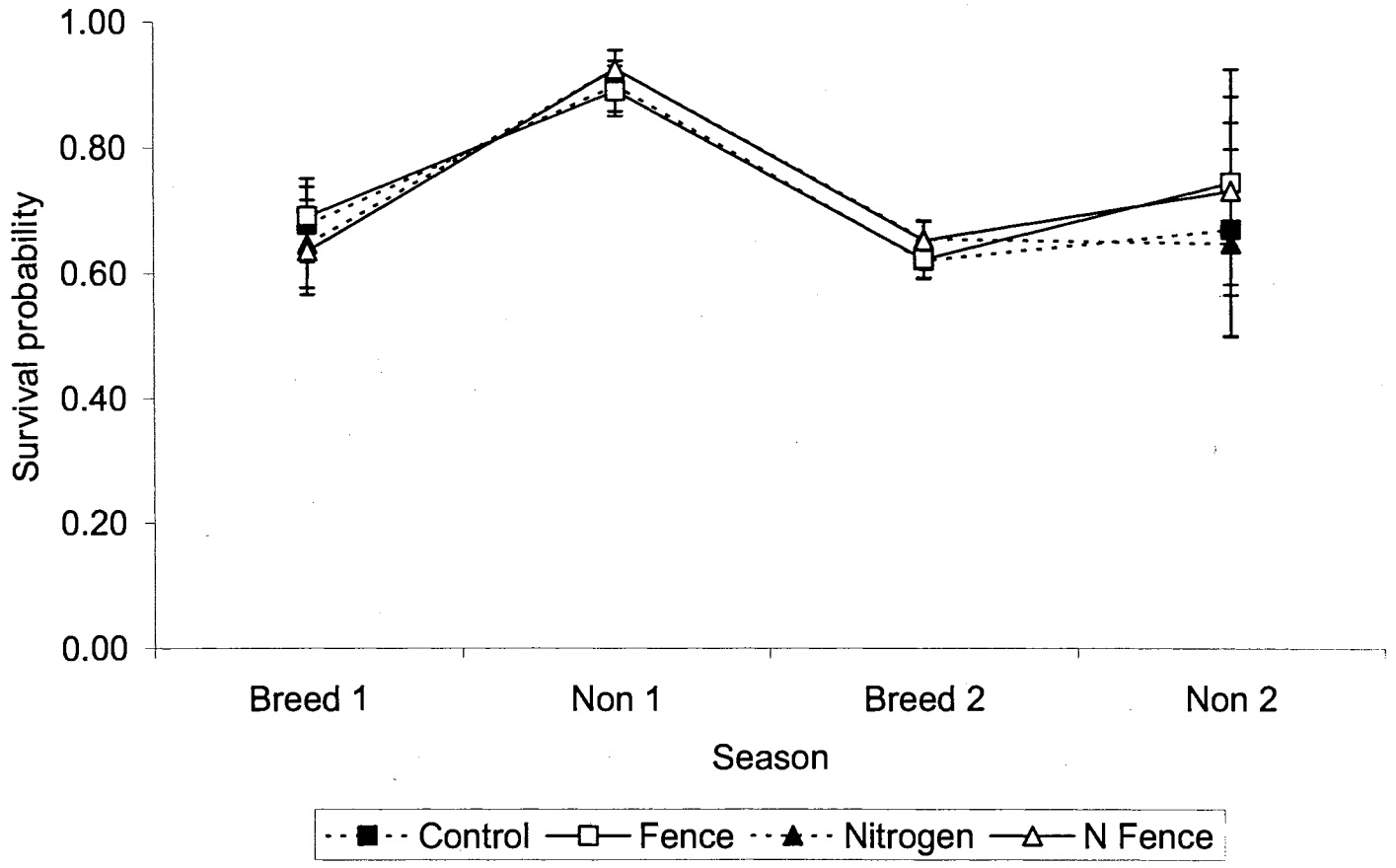
b)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Breed 2
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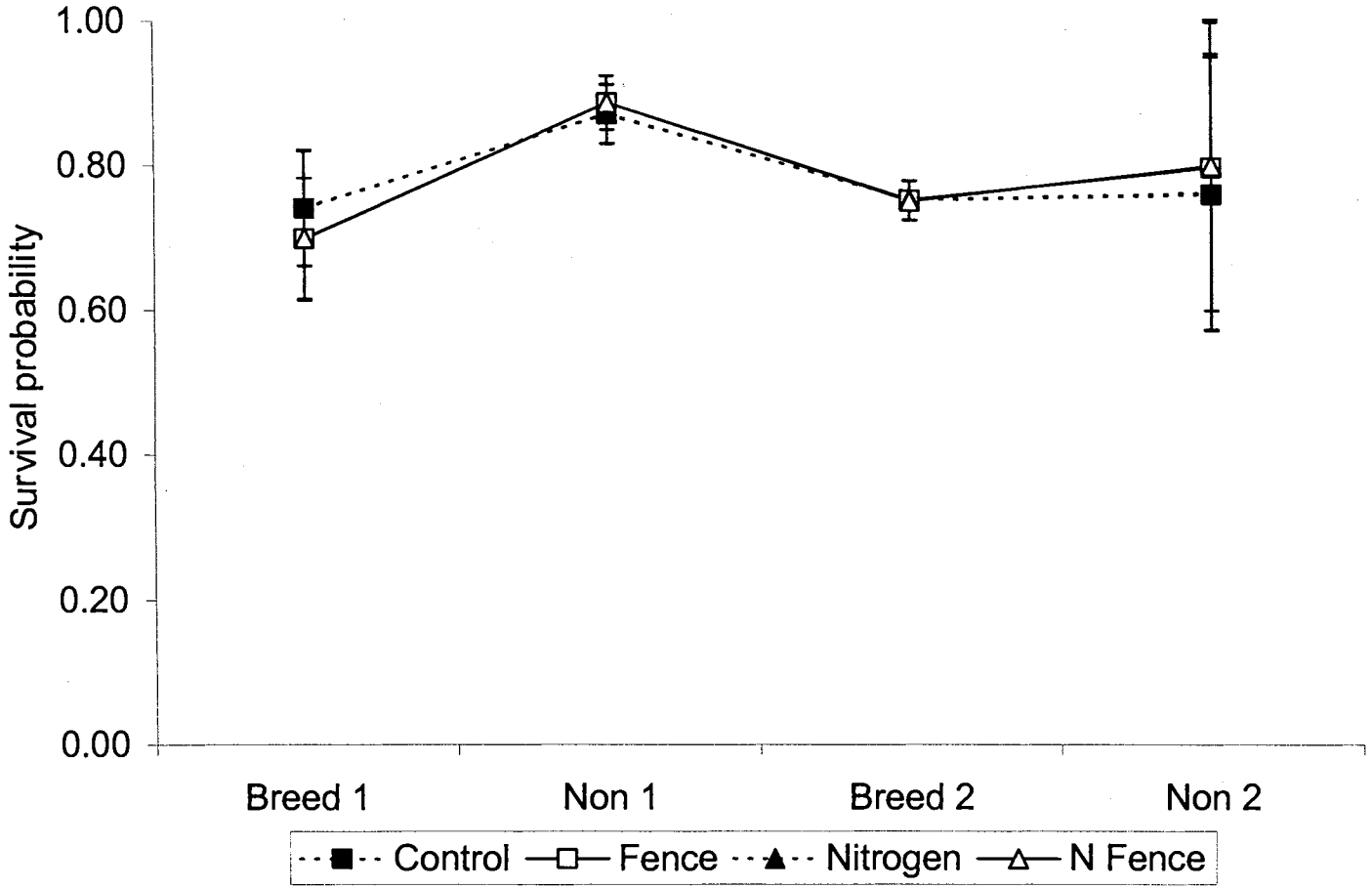
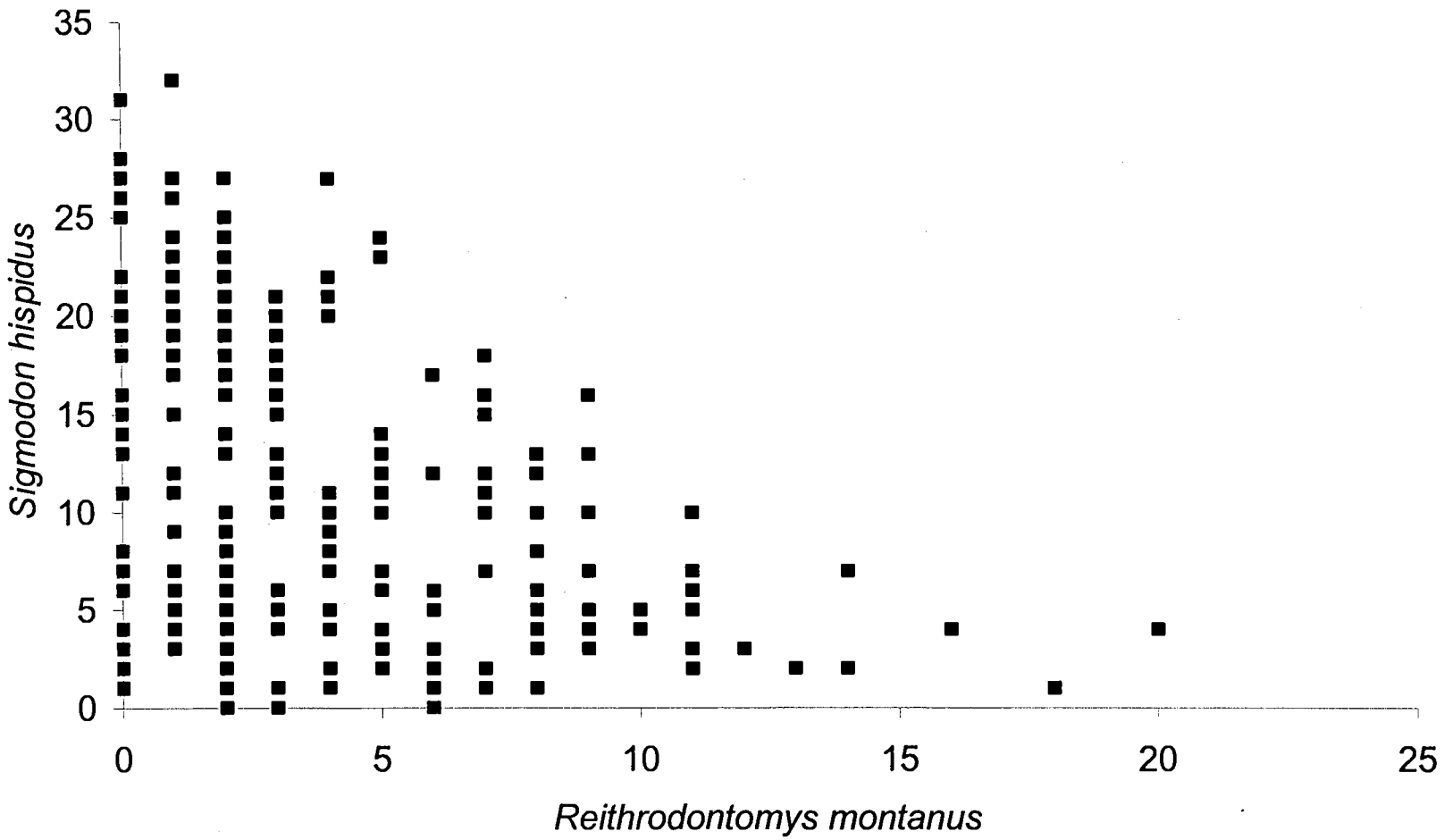
Nitrogen
N Fence


1.00
0.80
0.60
0.40
0.20
0.00
Survival probability
Breed 1
Non 1
Breed 2
Non 2
Control
Fence
Nitrogen
N Fence


35
30
25
20
15
10
5
0
Sigmodon hispidus
0
5
10
15
20
25
Reithrodontomys montanus

CHAPTER III

NITROGEN CONCENTRATION OF STOMACH CONTENTS AS AN INDEX OF DIETARY NITROGEN FOR HISPID COTTON RATS (*SIGMODON HISPIDUS*)

Abstract: We examined the reliability of using nitrogen concentration of stomach contents from hispid cotton rats (*Sigmodon hispidus*) as an index of dietary nitrogen. Stomach contents of hispid cotton rats fed pelleted diets varying in nitrogen concentration were analyzed for stomach nitrogen. Regression analysis revealed a positive linear relationship between stomach and dietary nitrogen, but the relationship was not 1:1. Thus, inverse estimation of the regression equation can be used to adjust for a lack of a 1:1 ratio and obtain more reliable and accurate estimates of diet quality. Although we expected this relationship to be robust to its application in field studies, model evaluation experiments with natural forages provided limited support for using our pelleted diet model to estimate dietary quality of cotton rats in field studies. The pelleted diet model consistently underestimated dietary nitrogen and was sensitive to estimates pertaining to cotton rats fed specific forages. We suggest developing multiple models with forages known to occur in the diet and at different stages of growth to account for variation in seasonal nitrogen availability and succulence of vegetation. We conclude that the applicability of using nitrogen concentration of the stomach contents of cotton rats as an index to dietary nitrogen is dependent on the level of accuracy and precision required in estimating nitrogen concentration of foods consumed.

INTRODUCTION

Availability of nitrogen-containing nutrients (e.g., protein) is an important factor in determining food quality for herbivores (White 1993). Therefore, estimates of nitrogen in diets of herbivorous mammals can assess quality of their food resources. Reliable estimation of dietary quality can provide insights into seasonal and annual variation in diet and habitat quality, requirements for maintenance and reproductive processes, and factors limiting distribution and abundance of animal populations.

Numerous methods have been used to assess dietary quality in small mammals. Analysis of forages known to occur in the diet of free-ranging small mammals are frequently used to estimate nutritional quality (Choo et al. 1981, Lindroth and Batzli 1984, Randolph et al. 1991). However, these analyses are subject to biases associated with selective feeding and may underestimate quality of food actually consumed (Sinclair et al. 1982, Peitz and Lochmiller 1993, Magomedov et al. 1996). Other potential biases from analyses of known forages include misidentification of forages consumed, temporal changes in quality of forages analyzed, and botanical diversity of diets (Peitz and Lochmiller 1993).

Indices of dietary quality from concentrations of fecal nitrogen have been widely advocated for herbivores (Leslie and Starkey 1985) and applied to a variety of wild ruminants (Leslie and Starkey 1985, Howery and Pfister 1990, Jenks et al. 1996, Osborn and Jenks 1998), hares (*Lepus* spp.; Sinclair et al. 1982, Magomedov et al. 1996), and rodents (Magomedov et al. 1996). Although indices of fecal nitrogen have been used to assess dietary quality in rodents, analysis of stomach contents has been suggested as a more reliable index (Magomedov et al. 1996).

Chemical analysis of stomach contents offers many advantages and eliminates biases associated with other techniques used to assess dietary quality in small mammals. For example, stomach contents provide an estimate of dietary quality that accounts for the relative proportion of each forage species in the diet (Servello et al. 1983). Analysis of stomach contents has been investigated in small mammals to estimate digestibility (Servello et al. 1983, MacPherson et al. 1985, MacPherson et al. 1988, Millar et al. 1991), nitrogen concentration (Bergeron and Jodoin 1994, Magomedov et al. 1996), and amino acid composition (Peitz and Lochmiller 1993) of the diet. We examined the relationship between nitrogen concentration of stomach contents from hispid cotton rats (*Sigmodon hispidus*) and the amount of crude protein (i.e., % nitrogen × 6.25) known to be in their diets. Our primary objective was to determine if the nitrogen concentration of stomach contents could be used as an index of dietary nitrogen. We created a model based on pelleted diets to examine this relationship. Although we predicted a positive relationship between nitrogen concentration in the diet and stomach contents, we did not expect this relationship to be 1:1 because of endogenous sources of protein found in the stomach (e.g., pepsin). Additionally, we tested our model with natural forage diets to determine if our model could accurately predict estimated nitrogen consumption from these diets.

METHODS

Laboratory procedures.— Our research colony was formed using wild-caught cotton rats trapped at various sites in Payne County, Oklahoma, using Sherman live-traps (7.6 by 8.9 by 22.9 cm), following standards established by the Animal Care and Use Committee of the American Society of Mammalogists (1998). Animals were housed at

the Laboratory Animal Resources facility at Oklahoma State University after capture at a temperature range of 20-25°C under 12L:12D. Cotton rats were housed individually in 48- by 25- by 20-cm wire-topped plastic cages with corn-cob bedding. We operated under Animal Care and Use Protocol 723, Oklahoma State University.

Cotton rats were bred and offspring raised to weaning (18 days; Parsons 2001). Weanling cotton rats were assigned randomly to 1 of 7 pelleted, isocaloric (18.0 - 18.4 kJ/g by formulation) experimental diets (Zeigler Brothers, Inc., Gardners, Pennsylvania) formulated to represent a range of nitrogen levels from 1.0–3.2%. Each diet was formulated identically except for relative amounts of soybean meal and corn meal, which were adjusted to achieve rations of 6, 8, 10, 12, 14, 16 and 20% crude protein (Table III.1). Diet formulations also included crystalline lysine and methionine to prevent these amino acids from becoming limiting, as they often are in corn-soy diets fed to monogastrics (D'Mello 1994).

Juvenile cotton rats were fed experimental rations until 6 weeks post-weaning at an age of 60 days. For the first 3 weeks post-weaning, they were housed in 28- by 18- by 13-cm plastic cages similar to those of adults. For the final 3 weeks of the feeding trial, juveniles were moved to larger cages (48 by 25 by 20 cm). Water and experimental diets were offered ad libitum throughout the entire 6-week period.

Each cotton rat was put under general anesthesia with Metofane (methoxyflurane, Mallinckrodt Veterinary, Inc., Mundeleine, Illinois) and euthanized via cervical dislocation at 6 weeks post-weaning. All animals were terminated at the same time of day (0800) to preclude influences of daily fluctuations in gut fill and water intake. Immediately after termination, the peritoneal cavity was opened and the stomach was

removed, cutting the stomach from the esophagus at the cardiac sphincter and from the proximal small intestine at the pyloric sphincter. The stomach was cut lengthwise along the lesser curvature, and contents were removed with a laboratory scoop (taking care to scoop out digesta without scraping the mucosa). Stomach contents were immediately placed in a labeled plastic test tube and frozen.

Stomach contents were freeze-dried (Model 77540 Freeze Drier, Labconco, Inc., Kansas City, Missouri) for 24 h, homogenized with a mortar and pestle, and weighed. Samples were analyzed for nitrogen content in duplicate with the macro-Kjeldahl technique (Foss Tecator 2400 Kjeltec Analyzer Unit, Foss North America, Eden Prairie, Minnesota). Stomach contents with a dry weight > 0.1g were considered for analysis. Samples also were taken from each experimental ration and ground in a mortar and pestle. Two sets of aliquots were taken simultaneously; 1 was oven-dried to constant mass (using duplicate samples) at 60°C to determine percent dry matter and the other was analyzed for nitrogen using the same technique as for stomach contents. Results of feed analyses subsequently were corrected for dry matter.

Statistical analysis.— We modeled the relationship between nitrogen content of the pelleted diets and stomach contents using simple linear regression (PROC REG; SAS Institute Inc. 2000). If a linear relationship existed, we examined whether the relationship was 1:1 by testing whether the slope was different than 1.0 (PROC REG; SAS Institute Inc. 2000). We performed diagnostic analyses using the residuals to investigate potential outliers and departures from linearity, constant variance, independence of observations, and normality. Additionally, we investigated use of the

dry weight of stomach contents for each rat as an independent variable (i.e., covariate) in a multiple regression equation to adjust the slope in the regression equation.

Forage Experiments.— We tested our model created from pelleted diets by conducting feeding trials with natural and agricultural forages. Cotton rats were bred and offspring were weaned at 18 days. After weaning, rats were offered a pelleted diet (A&M 20% Range and Breeder Cubes – Natural Protein, Stillwater Milling Company, Stillwater, OK) and water ad libitum and housed in 36- by 18.5- by 15-cm plastic cages (1–2 rats/cage) until the onset of the forage experiments. All rats used in the experiments were >60 g. During the experiments, rats were housed individually in 21- by 13- by 9-cm or 36- by 18.5- by 15-cm plastic cages. We assigned rats to 1 of 7 natural or agricultural forage diets: alfalfa (*Medicago sativa*), prairie hay composed primarily of Bermuda grass (*Cynodon dactylon*), German millet (*Setaria italica stramineofructa*) hay, white clover (*Trifolium repens*), or 1 of 3 samples of common wheat (*Triticum aestivum*) collected at 3 different locations during different stages of growth. The wheat-1 and -2 diets were collected prior to senescence (i.e., turning brown), whereas the wheat-3 diet was collected after some browning of the stems and leaves had occurred.

Three subsamples of each forage plant were analyzed for crude protein by the dry combustion method using a Leco CN-2000 carbon and nitrogen analyzer (Zhang et al. 1998) to estimate the mean nitrogen concentration of each diet. We weighed each individual forage sample before the experiment and offered forage samples and water ad libitum to experimental animals. Additionally, we weighed a subsample of each forage and oven-dried it to constant mass at 60°C for ≥48 h to estimate percent dry matter and moisture.

We euthanized each rat after of period of >40 h, removed the stomach, and collected stomach contents. All animals were killed between 0800–1100h. Preparation of stomach contents and subsequent nitrogen analyses were performed following procedures described in the pelleted diet experiment; however, samples containing <0.1 g dry weight were not analyzed in duplicate ($n = 9$). Additionally, orts were collected from each cage, oven-dried to constant mass at 60°C for ≥48 h, and analyzed for crude protein by the dry combustion method. Subsamples of each forage and orts from each rat were analyzed for crude protein at the Soil, Water and Forage Analytical Laboratory at Oklahoma State University. Percent dietary nitrogen (N), hereafter referenced as known dietary nitrogen, was measured for each rat by subtracting the amount of crude protein (g) in the orts from the total amount of crude protein offered and adjusting for dry matter consumed.

Model Evaluation.— We evaluated the predictive capabilities of our pelleted diet model using the inverse estimation of the linear regression model (i.e., calibration; Graybill and Iyer 1994:425). Therefore, we used our model to predict percent dietary nitrogen ($\hat{x}_0$), hereafter referenced as predicted dietary nitrogen, for rats fed forage diets as a function of the percent nitrogen concentration of stomach contents (y) by solving for dietary nitrogen in the linear regression equation

$$\hat{x}_0 = \frac{y_0 - \hat{\beta}_0}{\hat{\beta}_1},$$

where y_0 is the observed value of y, and $\hat{\beta}_0$ and $\hat{\beta}_1$ are estimates of the intercept and slope, respectively. A confidence interval for $\hat{x}_o$ can be computed following Graybill and Iyer (1993:429). To investigate the predictability of our model, we calculated the

mean and 95% confidence interval for the difference ($\hat{d}$) between known dietary nitrogen and predicted dietary nitrogen (i.e., $\hat{d} = N - \hat{x}_0$) to determine whether our model provided an unbiased estimate of dietary quality. Additionally, we calculated the mean and 95% confidence interval for the absolute difference ($\hat{d}_{ABS}$) to determine the average error in our estimate of dietary quality (i.e., $\hat{d}_{ABS} = |N - \hat{x}_0|$).

Post-hoc analyses.— The analyses described above were true *a priori* analyses. The following analyses were performed following detailed investigation and identification of patterns prevalent in the data and represent exploratory analyses.

We modeled the relationship between nitrogen content of the forage diets and stomach contents using simple linear regression (PROC REG; SAS Institute Inc. 2000). We performed 2 separate analyses because we identified values from 2 of the forage diets (wheat-2 and clover) as outliers. One regression analysis included all data from the forage trials and the other did not include data from rats assigned to the wheat-2 or clover diets. We performed diagnostic analyses for both models using residuals to investigate potential outliers and departures from linearity, constant variance, independence of observations, and normality. Transformations were investigated when necessary.

RESULTS

Pelleted diet model.— Stomach contents from 57 individual cotton rats fed pelleted diets were analyzed for nitrogen content. A positive linear relationship existed between nitrogen concentration of the stomach contents and diet [% stomach nitrogen = 0.67 + 0.82(% dietary nitrogen); $r^2 = 0.81$, d.f. = 55, $P < 0.001$; Fig. III.1]. However, the slope ($b_1 = 0.82 \pm 0.05$) from the regression analysis was different than 1.0 ($F = 11.55$, d.f. =1, 55, $P = 0.001$). Diagnostic procedures did not reveal any departures from model

assumptions, but identified 1 observation as a potential outlier. We investigated potential effects of this observation on our results and found no influences that would change our inferences from the data. Thus, we included the observation in all analyses.

Dry mass of the stomach contents for individual rats ranged from 0.15 - 1.80 g ($\bar{x}$ = 0.51, S.E. = 0.05). The addition of dry mass in the regression equation had little effect on the estimated slope (b_1 = 0.82 ± 0.05; stomach nitrogen = 0.69 + 0.82(dietary nitrogen) – 0.03(dry mass); r^2 = 0.81) and did not influence the relationship between stomach and dietary nitrogen. Thus, our model was created using the simple linear regression model above.

Model Evaluation with forages.— Stomach contents from 41 individual cotton rats fed natural or agricultural forage diets were analyzed for nitrogen content. Average nitrogen content of forages ranged from 0.72 to 5.04 % N (Table III.2) and nitrogen consumption (i.e., known dietary nitrogen) for rats on forage diets ranged from 0.23 to 5.37 % N. Although some measures of known dietary nitrogen (*N*) were outside the range of nitrogen concentrations of the pelleted diets (1.07–3.67 % N), we used only values of known dietary nitrogen that were within range of the nitrogen concentrations of our pelleted diets to evaluate the model (Table III.3). Values within this range corresponded to rats fed a wheat diet or Bermuda hay. Our pelleted diet model (inverse regression: $\hat{x}_o = (y_0 - 0.67) / 0.82$) consistently underestimated predicted dietary nitrogen; thus, the 95% confidence interval of the difference between known dietary nitrogen (*N*) and predicted dietary nitrogen ($\hat{x}_o$) did not include 0 ($\hat{d}$ = 0.86 ± 0.22, 95% CI = 0.40–1.32; Table III.3; Fig. III.2). The average error ($\hat{d}_{ABS}$) between known dietary nitrogen and predicted dietary nitrogen was 0.97 (± 0.20, 95% CI = 0.55–1.38).

Forage Models.— Post-hoc examination of the natural forage data revealed increased variation in the relationship between stomach and dietary nitrogen concentration as dietary nitrogen increased (Fig. III.3); however, much of the variation appeared to be attributed to 2 diets (wheat-2 and clover). Thus, we created 2 regression models to examine the relationship of stomach and dietary nitrogen using natural forage diets.

The model including all forage data did not meet the assumption of constant variance; thus, we used the inverse transformation of the dependent variable (i.e., nitrogen content of the stomach contents). The model with the inverse transformed data exhibited a negative relationship [1/stomach nitrogen = 1.18 – 0.17(dietary nitrogen), SE of the slope = 0.03, d.f. = 40, $P < 0.001$] and explained 43% of the variation in the data.

The regression model that did not incorporate data from rats fed the clover or wheat-2 diet did not require a transformation and had a positive relationship [stomach nitrogen = 0.37 + 0.82(dietary nitrogen), SE of the slope = 0.06, $r^2 = 0.88$, d.f. = 31, $P < 0.001$; Fig. III.3]. Furthermore, the slope of this model was similar to the slope of the pelleted diet model ($b_1 = 0.82 \pm 0.05$).

DISCUSSION

Our initial model from pelleted diets provided evidence for using nitrogen concentration of the stomach contents as an index to dietary quality for cotton rats. We observed a strong positive relationship between the nitrogen concentration of the stomach contents and known diets with 81% of the variation explained by the regression model. Similar to our study, Magomedov et al. (1996) examined the use of stomach contents to estimate the qualitative composition of food consumed by 6 rodent species and the

European hare (*Lepus europaeus*). They concluded that the chemical composition of the stomach contents almost entirely reflected the composition of food consumed with respect to protein (Magomedov et al. 1996). However, it is important to note that the relationship between nitrogen concentration in the stomach and known diets was not 1:1 in our pelleted model.

Presence of endogenous stomach nitrogen in the form of digestive enzymes and sloughed mucosal cells has 2 effects on the estimation of dietary quality from stomach contents. First, all measurements of ingested crude protein are elevated, resulting in the intercept being >0. Second, the response of stomach nitrogen to dietary nitrogen is altered by differential dilution of ingesta. At low levels of dietary nitrogen, a larger proportion of the crude protein measured in the stomach is derived from endogenous sources. At high nitrogen intakes, the ratio between ingested and endogenous nitrogen is much higher, and a closer relationship exists between dietary and stomach crude protein. A similar relationship is seen with apparent digestibility of protein (Robbins 1993:294). The result is a "flattening" of the relationship between these 2 variables and a slope <1.0. Our analysis and subsequent regression model for the pelleted diets corrected for these confounding influences and provided a more reliable and accurate estimate of dietary nitrogen.

Regression equations based on chemical analyses of stomach contents from voles *(Microtus)* fed known diets in the laboratory have been used to estimate digestibility of diets in wild populations (Servello et al. 1983, MacPherson et al. 1985). Our rationale for using regression analysis was not only to investigate reliability of using nitrogen concentration of the stomach contents as an index to dietary quality, but also to provide a

means to estimate dietary nitrogen in future studies of wild populations of cotton rats. Reliable estimates and confidence intervals of dietary nitrogen from stomach contents of wild populations can be obtained from the inverse estimation of the linear regression model (i.e., calibration; Graybill and Iyer 1994:425); therefore, making it possible to predict dietary quality as a function of stomach contents.

Studies investigating digestibility or nutrient consumption of small mammals from the analysis of stomach contents often use homogenized pelleted diets to formulate models or draw inferences that are then assumed applicable to examining these relationships in field studies (Servello et al. 1983, MacPherson et al. 1985, Peitz and Lochmiller 1993). Thus, it is assumed that relationships in these models hold for natural forages. Similarly, we expected this relationship to be robust in its application to field studies but realize the derived relationship in our pelleted diet model says nothing about nitrogen availability, which may vary substantially in foods with the same nitrogen content. For example, coniferous browses and leaves of many trees and shrubs contain tannins that reduce apparent digestibility of protein relative to grasses and legumes (Robbins et al. 1987, 1991). Because cotton rats feed primarily on low-tannin grasses and forbs (Randolph et al. 1991), we suspected an index of dietary nitrogen developed from pelleted diets likely would be positively correlated with protein availability in field studies.

Model evaluation experiments with natural forages provided limited support for using our pelleted diet model to estimate dietary quality of cotton rats in field studies. The pelleted diet model consistently underestimated dietary nitrogen and was sensitive to estimates pertaining to rats fed the wheat-2 diet (Table III.3; Fig. III.2). When data from

the wheat-2 diet were removed from the difference analyses, the means and 95% confidence intervals for the difference ($\hat{d}_{W2}$) and absolute difference ($\hat{d}_{ABSW2}$) between known dietary nitrogen and predicted dietary nitrogen were more accurate and precise ($\hat{d}_{W2} = 0.36 \pm 0.12$, 95% CI = 0.10–0.61; $\hat{d}_{ABSW2} = 0.49 \pm 0.08$, 95% CI = 0.32–0.67). Thus, deficiencies in accuracy and overall precision of the predicted estimates were largely attributed to the wheat-2 diet.

Why did our model based on pelleted diets underestimate nitrogen in forage diets? We posit that the main reason was differences in passage rate dynamics between the 2 types of foods. A similar mechanism was hypothesized by Servello et al. (1983) to account for differences in nutritional quality between stomach and diet contents in pine voles (*Microtus pinetorum*). As ingested pellets are reduced in size in the stomach by enzymatic, mechanical, and chemical processes, small particles passing into the small intestine and larger particles being retained in the stomach are similar in nutrient composition because of the homogeneity of pelleted diets. Conversely, natural forages are highly heterogeneous. In ruminants, breakdown rates of plant particles were inversely related to cell wall thickness and content (Spalinger and Robbins 1986). In some plants, highly digestible plant parts (which are commonly high in nitrogen) appeared to disintegrate more rapidly in the rumen, leaving behind more resistant, less digestible parts (Spalinger and Robbins 1986). Similar processes occurring in a monogastric stomach coupled with rapid overall passage rates (as low as 4 hours in highly succulent foods; Reid and Brooks 1994), would result in an increased likelihood of collecting stomach samples that are lower in nutritional quality, including nitrogen, than the fed-diet sample. In our *post-hoc* analyses, nitrogen content of the wheat-2 and clover diets were

underestimated the most (Fig. III.3), and these forages may have had the most heterogeneity in composition because of the combination of structural and actively-growing tissues. This postulate remains to be tested.

Other possible reasons for bias of our model include selective feeding by cotton rats, endogenous substances in the stomach, and time since feeding. Cotton rats may have fed on parts of plants that were lower in nitrogen, an unlikely scenario. In any case, we controlled for selective feeding by measuring nitrogen in fed forage and in orts, allowing us to adjust for actual nitrogen consumed. Endogenous substances in the mammalian stomach include hydrochloric acid, pepsin, gastrin, and mucus (Guyton and Hall 2001). The latter 3 of these compounds are proteins or protein-based and would elevate the nitrogen concentration of stomach contents, which is opposite to what we observed. Time since feeding may have affected our measure of nitrogen consumption and the predictive capabilities of our model; however, rats were euthanized during similar times of day to minimize influences of daily fluctuations in gut fill. Furthermore, time since feeding is impossible to determine in field application of our technique and requires assumptions similar to those we made in formulating and testing our model.

The inability to accurately estimate dietary quality in wild populations has been a recurring obstacle in assessing the role of nutrition in the ecology of small mammals. The applicability of using nitrogen concentration of the stomach contents of cotton rats as an index to dietary nitrogen is dependent on the level of accuracy and precision required in estimating nitrogen concentration of foods consumed. Our techniques for estimating dietary quality were based on simple models from laboratory trials that could be conducted for most species of small mammals and subsequently applied to wild

populations. However, we suggest developing such models with forages known to occur in the diet and at different stages of growth (i.e., during and after the growing season) to account for variation in seasonal nitrogen availability and succulence of vegetation. Therefore, it may be necessary to create more than one model to account for seasonal variation in digestibility. Future nutritional studies can use these techniques to gain more reliable estimates of dietary quality and its effects on the population ecology of small mammals.

ACKNOWLEDGMENTS

This project was funded by the United States Environmental Protection Agency (EPA) under an interagency agreement with the United States Geological Survey's Biological Resources Division and administered by the Oklahoma Cooperative Fish and Wildlife Research Unit (Oklahoma State University, Oklahoma Department of Wildlife Conservation, United States Geological Survey, and Wildlife Management Institute cooperating). The views expressed in this manuscript are those of the authors and do not necessarily reflect the views and policies of EPA. Additional funding for JEC was provided through a fellowship for the Environmental Institute at Oklahoma State University. We thank C. Bilder and M. Payton for statistical assistance and T. Bodine and H. Purvis for access to and assistance with laboratory equipment used in nitrogen analyses of stomach contents. We appreciate the assistance of M. Bahm, L. Newby, C. Powers, J. Lindsey, and P. Hanson in collecting data and secretarial assistance of J. Gray, H. Murray, and S. Lyon.

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Table III.1. Formulation of experimental rations (Zeigler Brothers, Gardners, Pennsylvania) differing in nitrogen concentration (N) for captive hispid cotton rats (*Sigmodon hispidus*).

	Percentage of each ingredient in diets						
Ingredient	0.96% N	1.28% N	1.60% N	1.92% N	2.24% N	2.56% N	3.20% N
Corn starch	54.88	50.82	46.77	42.71	38.66	34.61	26.50
Soybean meal	5.75	10.00	14.25	18.50	22.75	27.00	35.50
Cellulose fiber	13.50	13.50	13.50	13.50	13.50	13.50	13.50
Alfalfa	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soy oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcium phosphate	2.50	2.50	2.50	2.50	2.50	2.50	2.50

Table III.1. Continued.

Ingredient	Percentage of each ingredient in diets						
	0.96% N	1.28% N	1.60% N	1.92% N	2.24% N	2.56% N	3.20% N
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lignin sulfonate (pellet binder)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cane molasses	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine (98.5%)	1.16	1.00	0.83	0.67	0.50	0.33	-----
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Table III.1. Continued.

	Percentage of each ingredient in diets						
Ingredient	0.96% N	1.28% N	1.60% N	1.92% N	2.24% N	2.56% N	3.20% N
Choline chloride (70%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine (99%)	0.21	0.18	0.15	0.12	0.09	0.06	-----
% Nitrogen (by analysis)[a]	1.17 (0.05)	1.58 (0.03)	1.88 (0.05)	2.34 (0.10)	2.46 (0.04)	2.87 (0.02)	3.48 (0.07)

[a]Mean (*SE*).

Table III.2. Percent nitrogen (% N) and dry matter of natural forage diets fed to hispid cotton rats (*Sigmodon hispidus*). Wheat forages were collected at 3 different locations during different stages of growth.

Forage Species	% N of Forage[a]	% Dry Matter of Forage
Alfalfa	4.76 (0.15)	46.0
Bermuda hay	1.09 (0.06)	89.9
Millet hay	0.72 (0.01)	90.2
Wheat-1	2.18 (0.05)	35.2
Wheat-2	2.85 (0.14)	45.7
Wheat-3	1.30 (0.06)	62.6
White clover	5.04 (0.07)	42.2

[a]Mean (*SE*).

Table III.3. Predicted dietary nitrogen estimated from the pelleted diet model [stomach nitrogen = 0.67 + 0.82(dietary nitrogen)] for hispid cotton rats (*Sigmodon hispidus*) fed forage of known nitrogen content. Wheat forages were collected at 3 different locations during different stages of growth.

Rat ID	Forage Species	% N of Stomach Contents	Known % Dietary N (N)	Predicted % Dietary N[a] ($\hat{x}_0$)	Difference[b]
B3	Bermuda	1.11	1.07	0.53	0.53
B6	Bermuda	1.33	1.17	0.81	0.36
B7	Bermuda	1.35	1.01	0.83	0.18
B8	Bermuda	1.34	1.02	0.83	0.19
W1	Wheat 1	2.94	2.06	2.78	-0.72
W2	Wheat 1	2.05	1.92	1.68	0.23
W3	Wheat 1	1.83	1.95	1.42	0.54
W4	Wheat 1	1.68	1.91	1.24	0.68
W5	Wheat 1	2.29	2.17	1.99	0.18
W6	Wheat 1	2.66	2.05	2.43	-0.39
W7	Wheat 1	2.08	1.91	1.72	0.19
W8	Wheat 1	1.83	2.03	1.42	0.61
W62	Wheat 2	0.96	2.91	0.35	2.56
W63	Wheat 2	1.14	2.90	0.58	2.32
W64	Wheat 2	0.91	2.86	0.30	2.57

Table III.3. Continued

Rat ID	Forage Species	% N of Stomach Contents	Known % Dietary N (N)	Predicted % Dietary N[a] ($\hat{x}_0$)	Difference[b]
W65	Wheat 2	0.90	2.91	0.28	2.63
W66	Wheat 2	1.21	2.96	0.66	2.30
W71	Wheat 3	1.46	1.32	0.97	0.35
W72	Wheat 3	1.29	1.24	0.76	0.49
W73	Wheat 3	1.00	1.15	0.40	0.76
W74	Wheat 3	0.73	1.57	0.07	1.50

[a] Calculated using the inverse regression equation from the pelleted diet model to predict dietary quality ($\hat{x}_0$)

$$\hat{x}_0 = \frac{y_0 - \hat{\beta}_0}{\hat{\beta}_1},$$

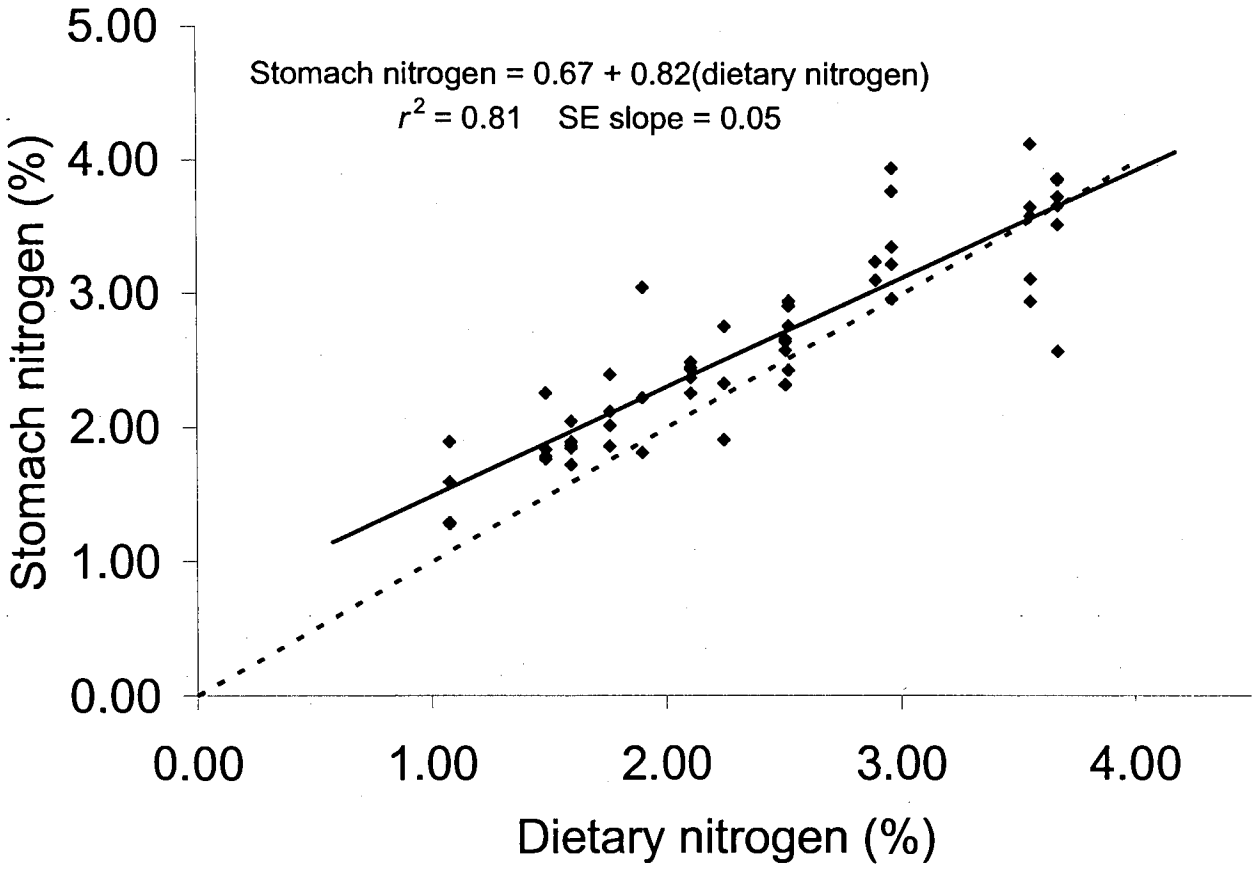
where y_0 is the observed value of y, and $\hat{\beta}_0$ and $\hat{\beta}_1$ are estimates of the intercept and slope, respectively.

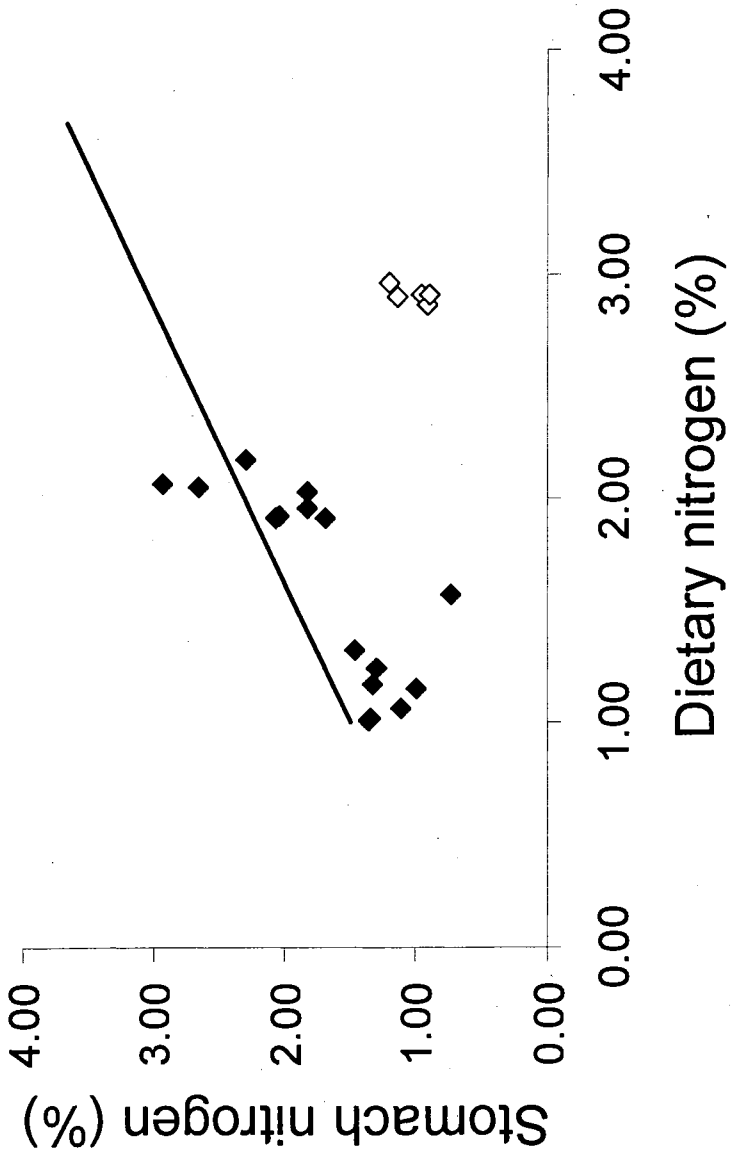
[b] Difference = (Known Dietary N – Predicted Dietary N).

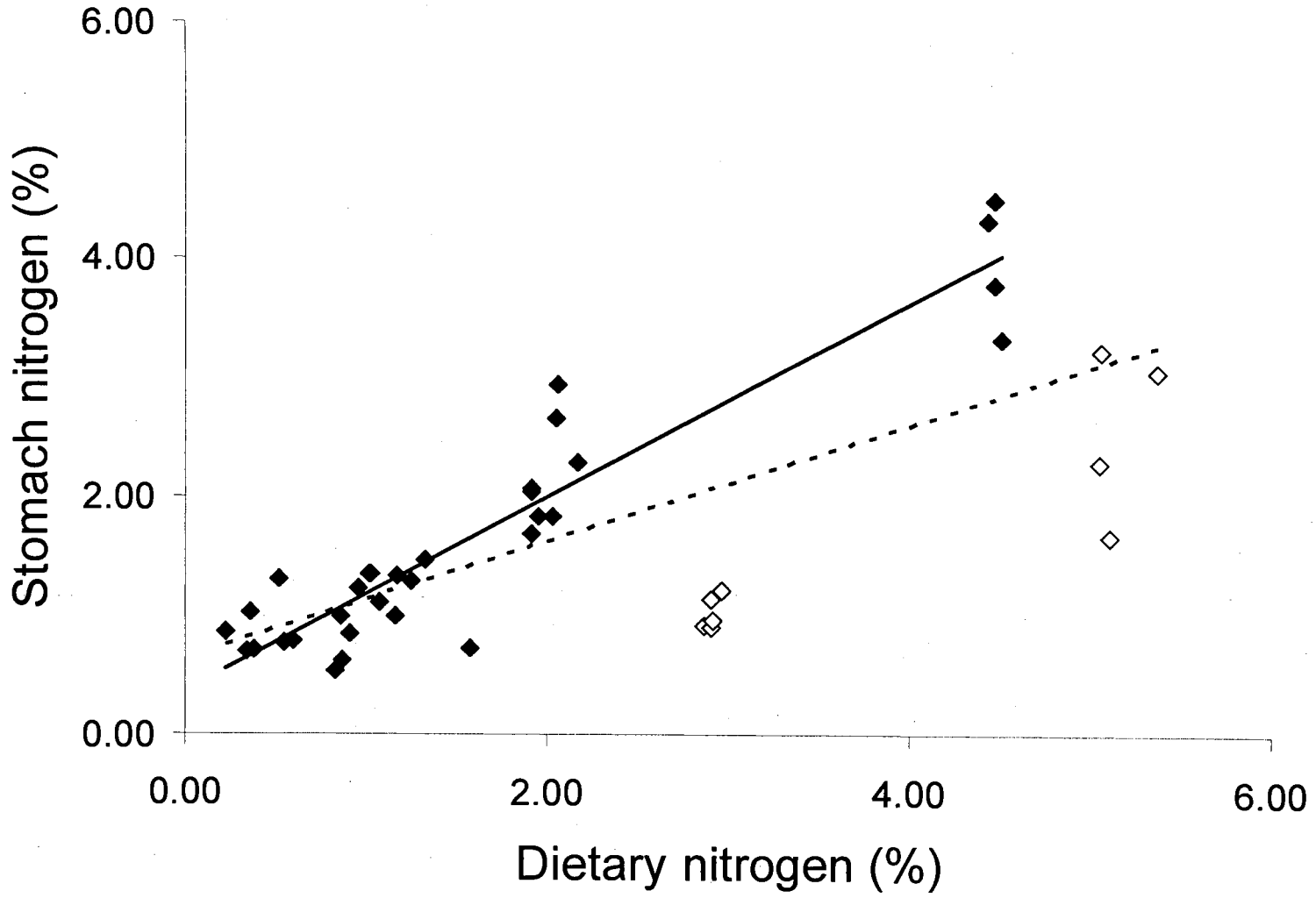
Fig. III.1. The observed (—) and 1:1 (---) relationships between nitrogen concentration in the stomach contents of hispid cotton rats (*Sigmodon hispidus*) fed pelleted diets of known nitrogen concentration.

Fig. III.2. Observed values of nitrogen concentration in the stomach contents at known levels of dietary nitrogen from hispid cotton rats (*Sigmodon hispidus*) fed natural forage diets and the predicted relationship(—) from the pelleted diet model ($\hat{x}_o = (y_0 - 0.67) / 0.82$). Only measures of known dietary nitrogen within the range of nitrogen concentrations used to create the pelleted diet model (1.07–3.67% N) were included in the analysis. Open diamonds correspond to rats assigned to the wheat-2 diet.

Fig. III.3. The relationships between nitrogen concentration in the stomach contents and known levels of dietary nitrogen of hispid cotton rats (*Sigmodon hispidus*) fed natural forage diets. The dashed trendline represents the graphical relationship among all diets (---); however, the estimated regression model required an inverse transformation [1/stomach nitrogen = 1.18 – 0.17(dietary nitrogen); SE slope = 0.03, $r^2 = 0.43$]. The solid trendline excludes data from the wheat-2 and white clover diets (—) and corresponds to the estimated regression model (stomach nitrogen = 0.37 + 0.82(dietary nitrogen); SE slope = 0.06, $r^2 = 0.88$). Open diamonds correspond to rats fed the wheat-2 or white clover diet.


Stomach nitrogen = 0.67 + 0.82(dietary nitrogen)
r^2 = 0.81 SE slope = 0.05
Stomach nitrogen (%)
5.00
4.00
3.00
2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
Dietary nitrogen (%)


Stomach nitrogen (%)
4.00
3.00
2.00
1.00
0.00
0.00
1.00
2.00
3.00
4.00
Dietary nitrogen (%)


6.00
4.00
2.00
0.00
Stomach nitrogen (%)
0.00
2.00
4.00
6.00
Dietary nitrogen (%)

CHAPTER IV

NITROGEN OUTPUTS OF SMALL MAMMALS FROM FECAL AND URINE DEPOSITION: IMPLICATIONS FOR NITROGEN CYCLING?

Abstract: The contribution of small mammals to nitrogen cycling is poorly understood, but it could have reverberations back to the producer community by maintaining or even magnifying nitrogen availability. Our objective was to model nitrogen outputs (deposition of feces and urine) of small mammals in an old-field ecosystem and estimate the amount of fecal and urinary nitrogen deposited annually. To address this objective, we used models from laboratory studies combined with data from field studies to estimate dietary nitrogen and daily and annual nitrogen outputs from fecal and urine deposition of 5 small-mammal species at the Environmental Protection Agency's Center for Subsurface and Ecological Assessment Research (CSEAR) during 2000. The models accounted for monthly fluctuations in density and average body mass of small-mammal populations. We estimated that small mammals deposited 1.15 and 3.21 kg $N \cdot ha^{-1} \cdot yr^{-1}$ from feces and urine, respectively, for a total contribution of 4.36 kg $N \cdot ha^{-1} \cdot yr^{-1}$. Hispid cotton rats (*Sigmodon hispidus*) accounted for >75% of the total nitrogen outputs by small mammals. Our estimates of annual fecal and urinary nitrogen deposited by small mammals were comparable to nitrogen deposits by larger herbivores and other nitrogen fluxes in grassland ecosystems.

Introduction

Herbivores have a variety of direct and indirect effects on plant communities (Huntly 1991, Davidson 1993, Jefferies et al. 1994, Ritchie et al. 1998) and may indirectly control form and function of ecosystems (Pastor and Naiman 1992, Jones and Lawton 1994, Lawton 1994). These include direct effects on plants by herbivory and deposition of feces and urine (McNaughton 1985) and indirect effects on plant community composition by seed dispersal and soil impacts (Gessaman and MacMahon 1984, Heske et al. 1994).

Deposition of urine and feces by herbivores significantly contributes to cycling of nitrogen and other nutrients in the ecosystem. The effect of fecal and urine deposition on vegetation is well documented for various herbivores, such as lesser snow geese (*Anser caerulescens caerulescens*; Bazely and Jefferies 1985, Ruess et al. 1989, Wilson and Jefferies 1996, Wilson et al. 1999), American bison (*Bison bison*; Day and Delting 1990), wildebeest (*Connochaetes taurinus*; Ruess and McNaughton 1987, 1988), moose (*Alces alces*; Pastor et al. 1993), and other ungulates (Woodmansee 1978, McNaughton 1985, Steinauer and Collins 1995). Deposits by large or gregarious herbivores created patches of increased nitrogen availability (Jaramillo and Detling 1992*a,b*), increased rates of nutrient cycling (Woodmansee 1978, Floate 1981), altered species composition (Day and Detling 1990), and increased plant growth within deposition patches (Gessaman and MacMahon 1984).

Small mammals also may create "islands of disturbance within plant communities" through deposition of feces and urine (Gessaman and MacMahon 1984). Batzli (1975) stated that small-mammal deposition of urine and feces combined with

increased rates of decomposition could result in rapid accumulation of nutrients at the soil surface. However, studies of effects of small mammals on nutrient cycling from urine and feces deposition are mostly correlative and based on observations of enhanced plant growth in the presence of high densities of small mammals (Pastor et al. 1996).

Future atmospheric deposition of nitrogen from anthropogenic activities is expected to increase as the global human population and reliance upon fossil fuels increase (Galloway et al. 1994, U.S. Environmental Protection Agency 1995). Thus, the amount of unretained nitrogen cycling through ecosystems is likely to increase and can reasonably be expected to cause future environmental problems. The contribution of small mammals in nitrogen cycling is poorly understood and could have reverberations to the producer community by maintaining or even magnifying increased nitrogen availability (Vitousek 1994). In addition, the rapid turnover rates of nutrients in small-mammal feces may introduce different temporal and spatial scales to nutrient cycling (Pastor et al. 1996). Our objective was to model nitrogen outputs (deposition of feces and urine) of small mammals in an old-field ecosystem and estimate the amount of fecal and urinary nitrogen (kg $N \cdot ha^{-1} \cdot yr^{-1}$) deposited annually.

Study Area

Field research was conducted at the Environmental Protection Agency's Center for Subsurface and Ecological Assessment Research (CSEAR) near Garr Corner, Pontotoc County, Oklahoma. The study area was an old-field site composed of 16 square 0.16-ha experimental plots surrounded and separated from adjacent plots by a 5-m uninhabitable mowed strip (total area = 3.45 ha; Figure IV.1). The experimental design consisted of 2 treatments (exclosure fencing and nitrogen amendment) randomly applied

to the study plots in a 2 × 2 factorial with 4 replicates per treatment (fenced, nitrogen amendment; fenced, control; unfenced, nitrogen amendment; unfenced, control). Fenced plots were surrounded by a 2-m high, 2.5-cm chain-link fence that allowed free movement of small mammals between plots but restricted access by terrestrial predators and larger mammalian herbivores (e.g., lagomorphs, artiodactyls). To ensure free movement of small mammals between plots, 7.5-cm triangular holes were cut at ground level and spaced at 2-m intervals around fenced plots.

Beginning in February 1999, we added nitrogen fertilizer (34% ammonium nitrate) to nitrogen-amended plots at a rate of 3.1, 5.1, 4.1, and 4.1 kg $N \cdot ha^{-1}$ in February, May, August, and November, respectively. Nitrogen amendment rates corresponded to seasonal rainfall proportions for central Oklahoma. Oklahoma receives about 10 $kg \cdot ha^{-1} \cdot yr^{-1}$ of nitrate via atmospheric deposition (National Atmospheric Deposition Program (NRSP-3)/National Trends Network 2002). Nitrogen amendments of 16.4 $kg\ N \cdot ha^{-1} \cdot yr^{-1}$ were chosen for the study because rates of atmospheric deposition of nitrogen in excess of this occur in industrialized regions of the globe and are projected to increase for the foreseeable future (Brimblecombe and Stedman 1982, Galloway et al. 1994, U.S. Environmental Protection Agency 1995, Vitousek et al. 1997). Clark et al. (*in press*) sampled aboveground live-mass and canopy cover throughout the current study and reported differences in aboveground live-mass between nitrogen-amended and non-amended plots following initiation of nitrogen applications.

Methods

We parameterized our nitrogen output model with field data where possible. Clark et al. (*in press*) and Clark et al. (*in review*) described detailed methods used to

sample small mammals at CSEAR. We included 5 species of small mammals in nitrogen calculations: hispid cotton rat (*Sigmodon hispidus*), plains harvest mouse (*Reithrodontomys montanus*), fulvous harvest mouse (*Reithrodontomys fulvescens*), white-footed mouse (*Peromyscus leucopus*), and deer mouse (*Peromyscus maniculatus*). We used estimates of minimum number known alive (MNKA; Krebs 1966) for cotton rats (Clark et al. *in press*) and *Reithrodontomys* spp. (Clark et al. *in review*) as indices of abundance on each plot at each sampling period. Additionally, we used our trapping data to estimate the average mass of an individual for each species of small mammal on each plot at each sampling period. We pooled estimates of abundance and mass for *P. leucopus* and *P. maniculatus* because captures of both species were uncommon and their mass is similar.

Dietary nitrogen estimation.— We estimated dietary nitrogen in the field for cotton rats, which comprised >90% of small mammal biomass at CSEAR. We live-trapped wild cotton rats at various locations in Payne County, Oklahoma, using Sherman live traps (7.6 x 8.9 x 22.9 cm). Captured animals were returned to the laboratory and housed in cages with water only for a period of 24-30 hours to allow pre-capture digesta to be excreted. We transported rats to CSEAR and randomly assigned rats to small, temporary enclosures (1.8 × 1.8 × 0.6 m) randomly placed within the experimental plots in April, May, and August 2000. We placed 2 rats in each enclosure and moved enclosures to a new location on the study area daily. After an acclimation period (18–24 h), we collected animals from the enclosures with snap traps baited with the scent of peanut butter. We checked snap traps 1–2 times/h to ensure that captured animals did not spoil. If cotton rats were not collected in snap traps after a period of 2–3 h, they were

visually located within the enclosure, captured by hand, and killed via cervical dislocation. All cotton rats captured from enclosures were immediately taken to a laboratory at CSEAR where stomachs were removed by cutting the stomach from the esophagus at the cardiac sphincter and from the proximal small intestine. After removal, stomachs were frozen and transported to Oklahoma State University.

We collected stomach digesta from each cotton rat by cutting the stomach lengthwise along the lesser curvature and removing the contents with a laboratory scoop (taking care to scoop out only digesta without scraping the mucosa). After we removed helminthes from the stomach digesta, the remaining stomach contents were placed in a labeled plastic test tube and frozen. Stomach contents were subsequently freeze-dried (Model 77540 Freeze Drier, Labconco, Inc., Kansas City, Missouri) for 24 h, homogenized with a mortar and pestle, and weighed. Samples were analyzed for nitrogen content in duplicate with the macro-Kjeldahl technique (Foss Tecator 2400 Kjeltec Analyzer Unit, Foss North America, Eden Prairie, Minnesota). If individual cotton rats did not produce adequate amounts of stomach digesta for nitrogen analysis (i.e., >0.15 g), we composited stomach contents by sampling period and plot.

We estimated dietary nitrogen of cotton rats at CSEAR for each sampling period from the nitrogen concentration of the stomach contents using an inverse regression model:

$$D = \frac{m_0 - 0.67}{0.87} \quad (1)$$

where D was the estimate of percent dietary nitrogen (i.e., dietary quality), m_0 was a specified average percent of the nitrogen concentration of stomach contents, and 0.67 and 0.87 were estimates of the intercept and slope, respectively (Clark et al. *in press*). That

model was constructed from laboratory trials with known levels of nitrogen in the diet and accounted for endogenous sources of nitrogen in the stomach (Clark et al. *in press*). We obtained an estimate of dietary nitrogen from the literature for *Peromyscus leucopus* (4 % N; Derting and Hornung *in press*) and used this estimate for *R. fulvescens*, *R. montanus*, and *P. maniculatus* because diets of these species primarily comprise insects and seeds (Wilkins 1986, Stancampiano and Caire 1995).

Nitrogen-output models— Using our estimates of dietary nitrogen, we estimated field rates of urine and fecal nitrogen deposition from models derived in laboratory experiments of the relationship between nitrogen intake and outputs (Parsons 2001). We estimated nitrogen outputs from fecal deposition (F = mg fecal N•day^{-1}) for a single cotton rat, *Peromyscus* spp., and *Reithrodontomys* spp., respectively, as:

$$F = W_i^{0.75} [122.02 + (69.32 \times D)], \quad (2)$$

$$F = W_i^{0.75} [63.16 + (123.68 \times D)], \quad (3)$$

$$F = W_i^{0.75} [156.56 + (154.48 \times D)], \quad (4)$$

where W was the average body mass (kg) at trapping period i and D was the species-specific estimate of dietary nitrogen (Parsons 2001). Likewise, we estimated nitrogen outputs from urine deposition (U = mg urine N•day^{-1}) for a single cotton rat, *Peromyscus* spp., and *Reithrodontomys* spp., respectively, as:

$$U = W_i^{0.75} [-101.87 + (329.32 \times D)], \quad (5)$$

$$U = W_i^{0.75} [15.78 + (290.97 \times D)], \quad (6)$$

$$U = W_i^{0.75} [-118.05 + (623.84 \times D)], \quad (7)$$

where W and D were defined above. Using results from equations 2–7, we estimated monthly and annual nitrogen outputs on each plot for each species of small mammal as:

$$\text{kg N}\bullet\text{plot}^{-1}\bullet\text{yr}^{-1} = \frac{\left(\sum d_i \times F_i \times t_i\right)}{10^6} + \frac{\left(\sum d_i \times U_i \times t_i\right)}{10^6} \quad (8)$$

where d was the MNKA for a given species at trapping period i on a given plot and t was the number of days between trapping period i and i+1. We calculated the species-specific and overall contribution of small mammals for the entire study area by summing across plots and converted to kg N•ha^{-1} by dividing by 3.45 ha (i.e., area of the study area).

Results

Dietary nitrogen of cotton rats— We collected stomach contents from 52 cotton rats placed in temporary enclosures at CSEAR and obtained 30 estimates of dietary nitrogen (error between duplicates < 10.0%) using equation 1. We pooled estimates of dietary nitrogen across months because 95% confidence intervals overlapped (April = 2.69–3.67, May = 1.21–4.49, August = 1.21–4.49). Thus, we used the overall mean ($\bar{x}$ = 3.16, SE = 0.25) as our estimate of dietary nitrogen for cotton rats in the nitrogen output model.

Nitrogen output models— Estimates of fecal and urinary nitrogen outputs (kg N•ha^{-1}) of all species of small mammals within a given sampling interval ranged from 0.03 to 0.15 and 0.09 to 0.42, respectively (Figure IV.2). Estimates of annual output (kg N•ha^{-1}•yr^{-1}) for fecal, urinary, and total nitrogen of all small mammals were 1.15, 3.21, and 4.36, respectively. Cotton rats accounted for >75% of the total nitrogen outputs by small mammals (Figure IV.3), and nitrogen output peaked in fall concomitant with cotton rat densities (Clark et al. *in press*). Similarly, spatial distribution of nitrogen outputs was heterogeneous across our experimental plots and was largely dependent on distribution of cotton rats (Figure IV.1; Clark et al. *in press*).

Discussion

The contribution of small mammals to nitrogen cycling is unclear. Although it is generally assumed that small mammals contribute to nitrogen cycling within ecosystems (Hayward and Phillipson 1979, Inouye et al. 1987, Pastor et al. 1996), the actual impact is difficult to assess. Pastor et al. (1996) examined mineralization and fungal spore composition of fecal pellets of meadow voles (*Microtus pennsylvanicus*) and red-backed voles (*Clethrionomys gapperi*) in mixed deciduous-conifer forests of the Great Lakes region. They reported faster turnover rates of nutrients for microtine pellets than for moose pellets (Pastor et al. 1993) and suggested that effects on localized nutrient availability from small mammals are probably at least as significant as those of large mammals in the boreal forest ecosystem. Furthermore, the rapid turnover rates of nutrients in feces of small mammals may introduce different temporal and spatial scales to nutrient cycling. For example, densities of cotton rats at CSEAR increased rapidly from April 2000 (30.1 rats•ha^{-1}) into October 2000 (112.5 rats•ha^{-1}; Clark et al. *in press*); thus, large increases in population density during summer, coupled with a rapid fecal mineralization rate, may have resulted in higher plant-available nitrogen late in the growing season than would occur in the absence of cotton rat activity (Pastor et al. 1996).

How does our estimate of nitrogen outputs by small mammals (total N = 4.36 kg N•ha^{-1}•yr^{-1}) compare with other nitrogen fluxes in grassland ecosystems? Compared to estimates of other nitrogen fluxes in tallgrass prairie, our estimate of small-mammal outputs was equivalent to at least 87% of N_2 fixation (1–5 kg N•ha^{-1}•yr^{-1}), 9–11% of plant uptake of nitrogen (40–50 kg N•ha^{-1}•yr^{-1}), and 22–44% of atmospheric deposition of nitrogen (10–20 kg N•ha^{-1}•yr^{-1}) at Konza Prairie in Kansas (Blair et al. 1998). Our peak

nitrogen output of 8.5 kg N/ha/yr on one plot (Figure 1), corresponded to an equivalent of 37.6 kg/ha/yr if all nitrogen was available as or functionally equivalent to NO^{-3}. This is essentially equivalent to doses that occur in high nitrate deposition regions (National Atmospheric Deposition Program (NRSP-3)/National Trends Network 2002) and is well within the range of bioavailable nitrogen exposure where ecosystem effects are most pronounced (Wedin and Tilman 1996, Jorgensen et al. 2002). Additionally, our estimate of annual fecal and urinary nitrogen deposited by small mammals was within the range of estimates for larger herbivores (cattle: 1.3–4.7 kg $N \cdot ha^{-1} \cdot yr^{-1}$, Schimel 1986; elk: 0.0–14.1 kg $N \cdot ha^{-1} \cdot yr^{-1}$, Schoenecker et al. 2002). Thus, outputs of small mammals may represent a significant nitrogen flux and contribution to nitrogen cycling of grassland ecosystems.

Estimates of nitrogen outputs from small mammals on individual plots were heterogeneous across our study area (Figure IV.1). Clark et al. (*in press*) examined population abundance of cotton rats across the nitrogen-amended landscape at CSEAR and reported higher densities on fenced plots, especially nitrogen-fenced plots. Because cotton rats accounted for most of the nitrogen outputs from small mammals, the same patterns observed in Clark et al. (*in press*) were observed in the current study with regard to the treatments. Therefore, heterogeneity of nitrogen outputs across the landscape at the plot-level largely can be attributed to the distribution of cotton rats. At a finer scale, distribution of fecal and urine deposition within a plot may be concentrated at micro-sites (e.g., along runways) and create heterogeneity in nitrogen availability across each plot.

Our models were based on empirical data, but assumptions related to our estimates of dietary quality for cotton rats should be acknowledged. Our reasoning for

using temporary enclosures and non-resident cotton rats to assess dietary quality (i.e., nitrogen consumption) was to avoid interfering with an on-going study of population dynamics at CSEAR; thus, we did not want to remove resident animals from our study area. By using enclosures, we assumed nitrogen consumption by cotton rats placed in the enclosures reflected that of resident animals. Furthermore, our inverse regression model (equation 1; Clark et al. *in press*) was formulated from estimates of nitrogen consumption based on laboratory diets. Clark et al. (*in press*) tested this model with natural forage diets and found limitations to its applicability in field studies; however, estimates of nitrogen consumption in the current study were within the range of previously recorded estimates for cotton rats in central Oklahoma (2.6–4.3% nitrogen; Schetter et al. 1998).

We also assumed that percent nitrogen consumption (D in equations 2–7) for each species was constant throughout our trapping periods. Although our estimate of nitrogen consumption for cotton rats was an average of our samples from April, May, and August, it does not reflect potential temporal variation in dietary quality. For example, dietary quality likely is lower during dormant seasons (e.g., winter), which would cause variation in nitrogen consumption. However, Schetter et al. (1998) found that nitrogen content of cotton rat diets remained high (>2.5% N) throughout the year in central Oklahoma. Finally, our models did not account for a number of potentially important sources of nitrogen outputs, such as carcass decomposition and increased nitrogen availability from decomposition of plant clippings not consumed.

The contribution of small mammals to nitrogen cycling of grasslands could have implications in predicting future changes in ecosystem structure and function following nitrogen-mediated shifts in the small-mammal community. Two contrasting hypotheses

have been proposed pertaining to herbivore modification of feedback between dominant plant species and nutrient cycling. The hypothesis that herbivores indirectly decelerate nitrogen cycling assumes that herbivores selectively consume and decrease the abundance of plant species with nitrogen-rich tissues, thus reducing aboveground productivity and rate of nitrogen cycling (Tilman 1988, Leibold 1989, Pastor and Naiman 1992, Wilson and Agnew 1992, Wedin 1994, Ritchie et al. 1998). In contrast, the hypothesis that herbivores accelerate nitrogen cycling assumes that dominant nutrient-rich plants are tolerant of and not limited by herbivory (McNaughton 1976, 1985; Tilman 1982, 1988; DeAngelis et al. 1989, Ruess et al. 1989, Holland et al. 1992, Sterner 1994). Determining whether small mammals accelerate or decelerate nitrogen cycling in old-field ecosystems was beyond the scope of our study; however, our estimates of fecal and urinary nitrogen were based on models using empirical data and provided an important piece of the puzzle in assessing the contribution of small mammals to nitrogen cycling.

Acknowledgments

This project was funded by the United States Environmental Protection Agency (EPA) under Interagency Agreement DW14938554-01-0 with the United States Geological Survey's (USGS) Biological Resources Division and administered by the Oklahoma Cooperative Fish and Wildlife Research Unit (Oklahoma State University, Oklahoma Department of Wildlife Conservation, United States Geological Survey's Biological Resources Division, and Wildlife Management Institute cooperating). The views expressed in this manuscript are those of the authors and do not necessarily reflect the views and policies of EPA or USGS. Additional funding was provided through a fellowship from the Environmental Institute at Oklahoma State University. We

appreciate the lab and field assistance of J. Lindsey, P. Hanson, L. Levesque, M. Bahm, J. Bahm, M. Leslie, K. Brazil and L. Newby and logistical assistance of J. Gray, H. Murray, and S. Lyon. The contributions of the late R. L. Lochmiller were important to the genesis of this project.

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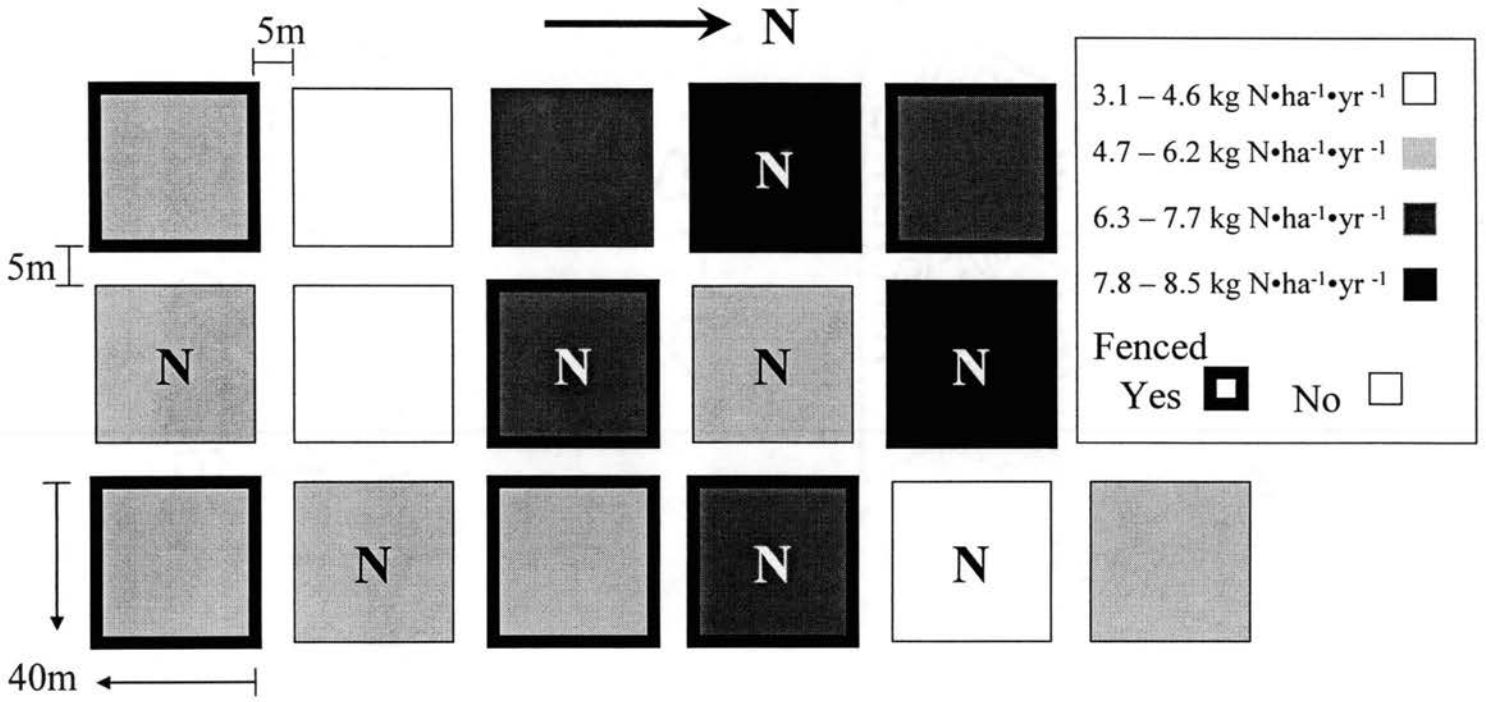
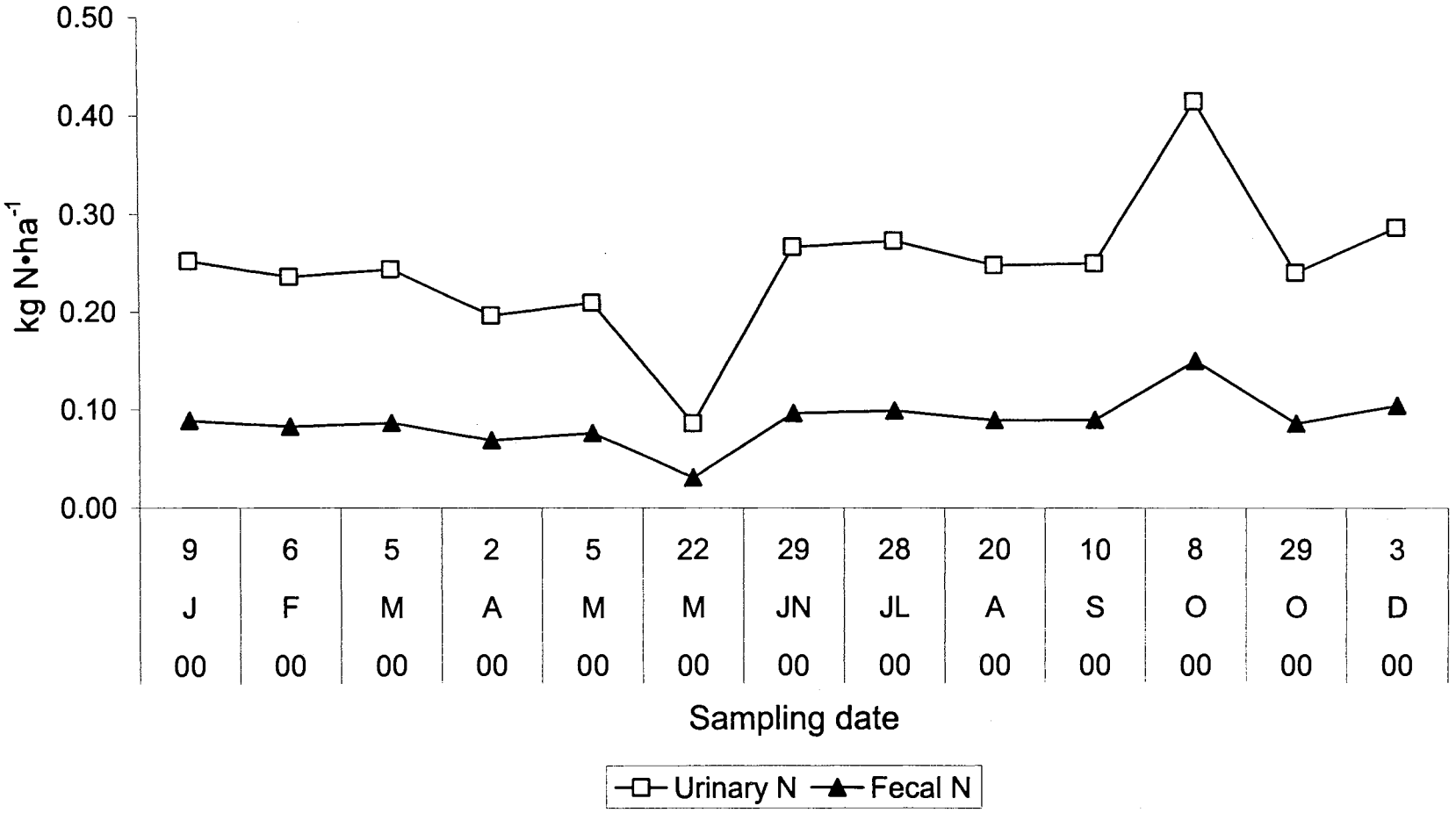
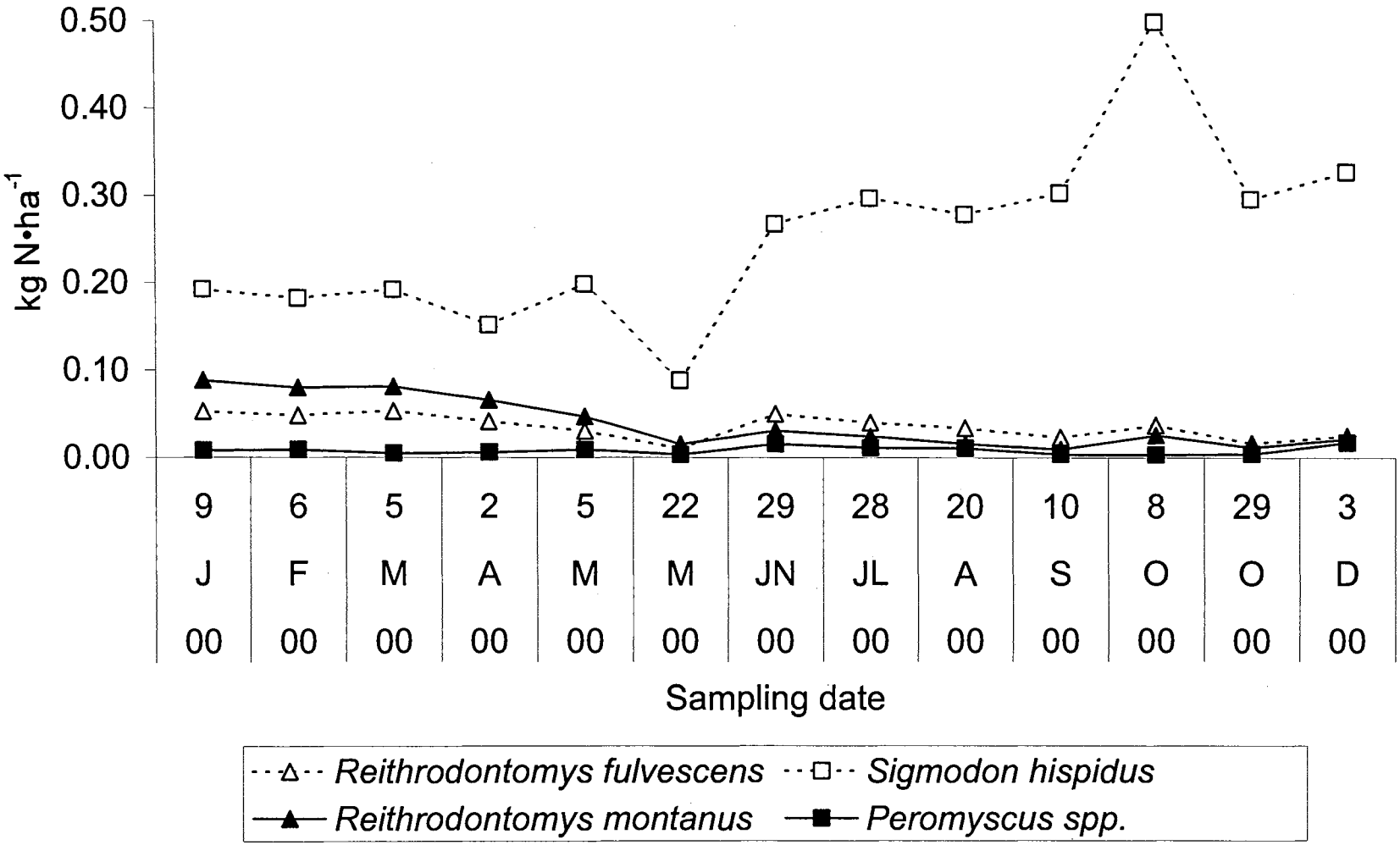
Figure IV.1. Layout of nitrogen (N) and exclosure treatments and spatial distribution of nitrogen outputs (i.e., fecal and urinary nitrogen) from small mammals across 0.16-ha plots at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 2000. Estimates of nitrogen outputs for each plot were extrapolated to kg $N \cdot ha^{-1} \cdot yr^{-1}$.

Figure IV.2. Monthly estimates of fecal and urinary nitrogen outputs (kg $N \cdot ha^{-1}$) of 5 species of small mammals at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 2000.

Figure IV.3. Monthly estimates of nitrogen outputs (kg $N \cdot ha^{-1}$) of hispid cotton rats (*Sigmodon hispidus*), fulvous harvest mice (*Reithrodontomys fulvescens*), plains harvest mice (*Reithrodontomys montanus*), and *Peromyscus* spp. (*P. leucopus* and *P. maniculatus* combined) at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 2000.


5m
N
5m
N
N
N
N
N
N
N
N
N
40m
3.1 – 4.6 kg N•ha-1•yr -1
4.7 – 6.2 kg N•ha-1•yr -1
6.3 – 7.7 kg N•ha-1•yr -1
7.8 – 8.5 kg N•ha-1•yr -1
Fenced
Yes
No


0.50
0.40
0.30
0.20
0.10
0.00
kg N•ha-1
9 J 00
6 F 00
5 M 00
2 A 00
5 M 00
22 M 00
29 JN 00
28 JL 00
20 A 00
10 S 00
8 O 00
29 O 00
3 D 00
Sampling date
Urinary N
Fecal N


0.50
0.40
0.30
0.20
0.10
0.00
kg N•ha-1
9 J 00
6 F 00
5 M 00
2 A 00
5 M 00
22 M 00
29 JN 00
28 JL 00
20 A 00
10 S 00
8 O 00
29 O 00
3 D 00
Sampling date
Reithrodontomys fulvescens
Sigmodon hispidus
Reithrodontomys montanus
Peromyscus spp.

CHAPTER V

CATASTROPHIC DECLINE OF A HIGH-DENSITY POPULATION OF HISPID COTTON RATS (*SIGMODON HISPIDUS*) IN CENTRAL OKLAHOMA

The geographic distribution of the hispid cotton rat (*Sigmodon hispidus*) extends northward from South America into the southern U.S. (Cameron and Spencer, 1981). Along the northern periphery of their range, populations of *S. hispidus* are vulnerable to major reductions in density and occasional local extinctions as a result of severe winter weather (Schendel, 1940; Cockrum, 1948; Goertz, 1964; Dunaway and Kaye, 1961; Fleharty et al., 1972; Sauer, 1985; Langley and Shure, 1988). We report on a high-density population of hispid cotton rats in central Oklahoma that declined to near extinction following consecutive severe winter weather events during December 2000. Furthermore, we present evidence of populations of hispid cotton rats being affected at a statewide scale as a result of the weather in December 2000.

The field research was conducted at the Environmental Protection Agency's Center for Subsurface and Ecological Assessment Research (CSEAR) near Garr Corner, Pontotoc County, Oklahoma. The study area was an old field composed of 16 square 0.16-ha plots surrounded and separated from adjacent plots by a 5-m mowed strip (total area = 3.45 ha). As part of an investigation of the role of small mammals in nitrogen cycling of old fields, 2 treatments (exclosure fencing and nitrogen addition) were applied to the study plots in a 2 × 2 factorial with 4 replicates per treatment combination. Fenced plots were surrounded by 2-m high, 2.5-cm chain-link fence that allowed free movement of small mammals between plots but restricted access to larger herbivores (e.g., lagomorphs, artiodactyls).

We sampled small mammals with Sherman live-traps (7.6 × 8.9 × 22.9 cm) for 3 consecutive days at 3-5-week intervals from July 1999 to July 2001 and at 3-month intervals thereafter. Each plot contained 25 traps systematically spaced at 7-m intervals for a total of 1,200 trap nights/sampling interval. Traps were baited with rolled oats, checked between 0600 and 1200 hours, and stuffed with cotton during cold weather (<10°C) to provide warmth for captured animals. We released captured animals immediately after marking with a unique number via toe-clipping. We used minimum number known alive (MNKA; Krebs, 1966) to estimate abundance of hispid cotton rats on the study area at each sampling period.

Between July 1999 and November 2001, we sampled at CSEAR 29 times and recorded 8,417 captures of small mammals in 34,800 trap nights. Hispid cotton rats accounted for 5,562 captures of 1,010 individuals. Density estimates ranged from 0.0 cotton rats/ha in July 1999 to 112.5 cotton rats/ha in October 2000 (Fig. V.1).

Populations of hispid cotton rats typically experience an annual bimodal density pattern in southern portions of their range with peaks in spring and autumn (Cameron 1977). However, densities in northern areas do not exhibit a bimodal pattern and typically have an autumn peak followed by a spring low (Fleharty et al. 1972). Although hispid cotton rats reproduce year round in warmer parts of their range, northern populations usually do not reproduce from November–March (Glass and Slade 1980, Stokes 1994, Wilson and Lochmiller 2002) and decline during winter (Stokes 1994). Densities of hispid cotton rats in our study were not bimodal and exhibited a pattern more similar to those reported for northern populations (Fig. V.1).

Between our sampling periods on 3 December 2000 and 14 January 2001, 3 independent winter weather events, in conjunction with the state's coldest month since 1983 (December 2000 statewide average temperature = -0.7°C; National Climatic Data Center 2000), affected central and eastern Oklahoma, including Pontotoc County. These winter storms resulted in accumulations of 2.5–5.0 cm of snow on 13 December, 2.5–5.0 cm of ice on 26 December, and >12.7 cm of snow on 31 December (National Climatic Data Center 2000). We recorded a drastic decline in the population of hispid cotton rats at CSEAR following these winter weather events. Densities dropped from 58.6 cotton rats/ha on 3 December 2000 to 1.2 cotton rats/ha on 14 January 2001 (Fig. V.1). Although hispid cotton rat densities were declining before these winter weather events occurred, we attributed the dramatic decrease to severe winter weather and below-normal temperatures. The ice accumulations on 26 December followed by snow on 31 December could have been especially damaging to the population because the layer of ice and snow persisted for >5 days and likely restricted foraging.

Langley and Shure (1988) attributed a crash of a population of hispid cotton rats in Georgia to extreme winter temperatures and spring-summer drought conditions. This population had not recovered after 22 months. As of 19 November 2001, the population of hispid cotton rats at CSEAR had not recovered. Abundances between January and November 2001 ranged between 0.6–2.6 cotton rats/ha compared with a range of 30.1–112.5 during the same period in 2000 (Fig. V.1).

Our study area had been grazed for >40 years before 1999. Thus, at the onset of our study, CSEAR contained little available habitat and hispid cotton rats were absent from our experimental plots. This initial absence of hispid cotton rats was not

unexpected, because Phillips (1936) had previously reported an absence of hispid cotton rats in overgrazed pastures in central Oklahoma. However, in the absence of grazing, habitat quality improved and hispid cotton rats colonized and increased rapidly via reproduction and immigration from surrounding areas. Unlike the manner in which the population of hispid cotton rats established in 1999, we attributed the slow recovery of the population during 2001 to the wide area affected by the weather events of December 2000 and subsequent lack of immigration from suitable habitats surrounding our study plots. Furthermore, reproduction of hispid cotton rats at CSEAR occurred primarily between March and October (J. E. Clark, unpublished data), thus increases in density from reproduction will not likely occur until at least summer 2002.

The winter weather events and below-normal temperatures of December 2000 appeared to affect not only the population of hispid cotton rats at CSEAR, but also populations in other areas of the state. Before December 2000, we frequently trapped an area in Payne County, Oklahoma (ca. 169 km N of CSEAR), to obtain hispid cotton rats for laboratory experiments and had capture successes as high as 20-30 captures/100 trap nights. In October 2001, we trapped the same area and had a capture success of 1.2 captures/100 trap nights (J. E. Clark, personal observation). Hispid cotton rats at the Tallgrass Prairie Preserve, Osage County, Oklahoma (ca. 241 km N of CSEAR) also experienced a dramatic decline in numbers. Capture success at the Tallgrass Prairie Preserve declined from 2.2 captures/100 trap nights in December 2000 to 0.0 captures/100 trap nights during monthly sampling periods from January to November 2001 (K. L. Soeder, Oklahoma State University, unpublished data).

Expansion of the geographic distribution of the hispid cotton rat has been documented along the northern periphery of its range (Cameron 1977). As hispid cotton rats extend northward, they are subjected to more pronounced changes in seasonal climates, leading to shorter breeding seasons and harsher winters. Thus, selective pressures related to reproductive output may be constrained by requirements for surviving harsher winters (Doonan and Slade, 1995). Although our observations were anecdotal and northern populations of hispid cotton rats tend to decline precipitously during winter (Stokes 1994), our conclusion regarding the drastic decline of the population at CSEAR was warranted given the magnitude of the decline, severity of the weather events of December 2000, and drastic reductions in other populations from Oklahoma. As suggested by Cockrum (1952), we suspect that severe winters, such as the events described above, may slow the northward advance of hispid cotton rats and serve to regulate populations along the intermediate and northern fringes of its range.

Acknowledgments

This project was funded by the United States Environmental Protection Agency (EPA) under an interagency agreement with the United States Geological Survey's Biological Resources Division and administered by the Oklahoma Cooperative Fish and Wildlife Research Unit (Oklahoma State University, Oklahoma Department of Wildlife Conservation, United States Geological Survey's Biological Resources Division, and Wildlife Management Institute cooperating). The views expressed in this manuscript are those of the authors and do not necessarily reflect the views and policies of EPA. Additional funding was provided through a fellowship from the Environmental Institute at Oklahoma State University. We thank K. L. Soeder for contributing data from the

Tallgrass Prairie Preserve and D. M. Leslie, Jr., and 2 anonymous reviewers for comments on an earlier draft of this manuscript. We thank S. Fox and S. Perea for the Spanish translation of our summary. We appreciate the field assistance of J. Lindsey, P. Hanson, L. Levesque, M. Bahm, M. Leslie, and K. Brazil and secretarial assistance of J. Gray, H. Murray, and S. Lyon.

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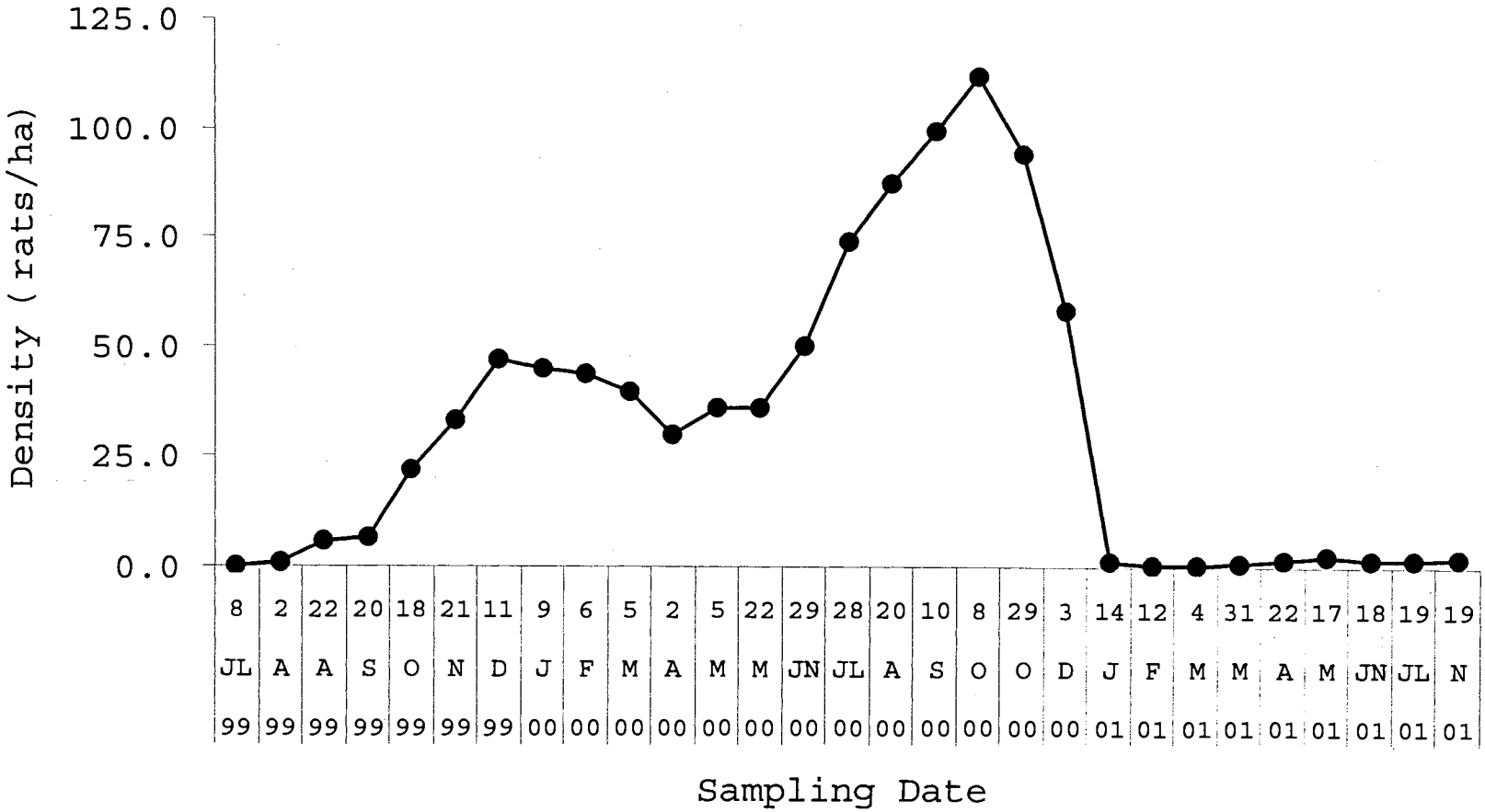
Fig. V.1. Density estimates of hispid cotton rats (*Sigmodon hispidus*) at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, July 1999 – November 2001.


125.0
100.0
75.0
50.0
25.0
0.0
Density (rats/ha)
8 JL 99
2 A 99
22 A 99
20 S 99
18 O 99
21 N 99
11 D 99
9 J 00
6 F 00
5 M 00
2 A 00
5 M 00
22 M 00
29 JN 00
28 JL 00
20 A 00
10 S 00
8 O 00
29 O 00
3 D 00
14 J 01
12 F 01
4 M 01
31 M 01
22 A 01
17 M 01
18 JN 01
19 JL 01
19 N 01
Sampling Date

#7

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